PLANTS

Fundamental and mission-oriented research is supported which provides basic understanding of mechanisms and processes of plant systems. This knowledge is necessary as a basis for developing management strategies for improving and sustaining agricultural production systems. Grants are awarded under five programs: Plant Genome, Plant Genetic Mechanisms, Plant Growth and Development, Photosynthesis and Respiration, and Nitrogen Fixation and Nitrogen Metabolism.

PLANT GENOME

Panel Manager - Dr. William D. Beavis, Pioneer Hi-Bred International
Program Director - Dr. Ed Kaleikau

This competitive grant program is part of the USDA Plant Genome Research Program, which was established to facilitate development of new or improved crop and forest species. The Program's goals are to promote sustainability and profitability of plant production, and to improve the quality of food, fiber, and feed. To accomplish these goals, the Program gives high priority to research for construction of genomic maps, and for detailed studies of specific regions of genomes, genes, or genetic processes. The program also supports research on development of new methods or innovative approaches that have potential application to create new germplasm/varieties, genome mapping, gene isolation, or gene transfer in crop and forest species.

9801281 Positional Cloning of a Major Gene from a Durably Resistant Rice Cultivar

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Grant 98-35300-6174; $150,000; 3 Years

Rice blast, an often devastating disease that occurs in most rice growing areas worldwide, costs farmers $5 billion a year. The use of resistant cultivars is the most economical and effective method of controlling the disease caused by *Pyricularia oryzae*. The traditional African rice cultivar Moroberekan has grown for more than twenty years in large areas without great losses from blast. The long term goal of this project is to gain an understanding of the molecular basis for durable resistance. We have shown in our previous studies that, although resistance in Moroberekan is genetically complex, much of its qualitative resistance is attributable to a single region of the rice genome. We have identified markers flanking this region and propose to isolate the region using a map-based cloning strategy. Direct transfer of this region to susceptible cultivars will provide an efficient method for utilizing a disease resistance gene(s) from a durably resistant cultivar. Molecular characterization of this region may also lead to an understanding of the mode of action of durable host resistance to fungal and other microbial pathogens.

9801286 Dissection and Transgenic Introgression of Disease Resistance Genes

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Grant 98-35300-6188; $395,000; 3 Years

Plants contain hundreds of probable disease resistance genes. Clusters of resistance genes are potential reservoirs of useful resistance specificities, some expressed and some cryptic. It is now easy to isolate numerous resistance gene candidates (RGCs) at the molecular level; however, it remains difficult to identify genes with individual recognition specificities that are agriculturally useful. We will develop and implement an efficient strategy to test the function of RGCs and to access resistance specificities from functional and cryptic genes in cultivated and wild genotypes. We will dissect the function and evolution of resistance genes in lettuce, as well as, generate agriculturally useful genotypes. Lettuce is a valuable vegetable crop and downy mildew is its most important disease. Lettuce is also an experimentally tractable and appropriate system for the characterization of disease resistance genes. We have characterized the largest cluster of resistance genes to date and developed new ideas as to how such clusters are evolving new specificities. We also have identified numerous wild accessories with resistance to all known isolates of downy mildew. We will create chimeric resistance genes by swapping regions closely and less-closely related RGCs to identify segments determining specificity and segments responsible for effector function. Based on this information, we will develop an expression cassette for the specificity segment(s) to test specificity regions using shotgun complementation. Genes expressing different resistance specificities to downy mildew will be pyramided and introduced to provided potentially more durable resistance.

9801296 Marker-assisted Introgression of General Resistance QTL for Late Blight in Tomato

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Grant 98-35300-6187; $250,000; 3 Years

The fungus *Phytophthora infestans* causes late blight disease on tomato plants and fruit, and is a major cause of crop loss. Fungicides do not adequately control this disease and are being eliminated due to environmental and consumer safety concerns. No adequate late blight resistance exists in cultivated tomato (*Lycopersicon esculentum*). Four genes (QTL) governing general resistance to late blight are being transferred from a wild tomato relative (*Lycopersicon hirsutum*) to cultivated tomato using DNA marker-assisted selection. Our laboratory had previously mapped the four resistance genes to the tomato chromosomes using RFLP and DNA markers. We will use DNA markers based on DNA amplification (PCR) to trace these resistance genes during transfer from wild to cultivated tomato in order to select those tomato lines that contain the desired resistance genes. Breeding lines with different combinations of the four resistance genes will be used to examine the effects of each gene on the expression of resistance. Cultivated tomato breeding lines with enhanced resistance to late blight, and the DNA markers associated with the resistance genes, will be released publicly for further breeding efforts.

9801175 Fine Mapping of Novel Nematode Resistance Clusters in Tomato
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Grant 98-35300-6350; $200,000; 3 Years

Root-knot nematodes cause major economic damage to many crops, including tomato. Currently only one nematode resistance gene, *Mi*, is present in cultivated tomato. The value of *Mi* has been compromised by the appearance at several locations in California and in other plants of the world of variants of nematode strains and species that can infect *Mi*-bearing tomato. *Mi* is also not effective at temperatures above 28°C. We have identified several new resistance genes in the wild tomato species *Lycopersicon peruvianum*. To linked loci are present in 2 different clones of the same accession of *L. peruvianum*. The long term goal of this project is to clone these resistance genes. We propose to develop linked markers to these genes and to identify their genetic map positions. We will develop a detailed map of these loci and identify the presence of resistance-gene homologues in these clusters. Besides enhancing our basic understanding of the structure of resistance gene loci, this study will have significant applied consequences as markers derived from these analyses will be available for use in public and commercial tomato breeding programs. We will characterize the haplotype of resistant germplasm to avoid the use of redundant sources of resistance. The markers will be integrated into our breeding efforts as they become available, to develop tomato genotypes with multiple resistance to root-knot nematodes.

9801156 Analysis and Cloning of Male-sterility Genes
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Grant 98-35300-6152; $175,000; 2 Years

The Green Revolution owed much of its success to the development of new hybrid lines of crop plants, superior to inbred lines of plants in vigor, disease-resistance and reproductive capacity. Hybrids are the result of the genetic crossing of two different parent plants. In order to make a hybrid, designated female plants must not self pollinate, which produces inferior inbred plants in the next generation. At this time, self-pollination of corn plants is prevented by physical removal of the male flowers (the source of pollen) at the top of the plant by a process called detasseling. Pollen is often provided by unrelated, designated male plants. Detasseling is a very labor-intensive process costing the corn seed industry millions of dollars yearly. Another approach to generate hybrids is to use plants that cannot produce pollen due to a genetic defect; these are called “male sterile” plants. The purpose of our proposal is to determine how many genes are present in the maize genome that, when mutated, produce male sterility. In addition, we are determining why these genes are required for producing mature pollen, both by analyzing the developing pollen in the mutants and by cloning the genes. We hope that our efforts will be used in the development of new ways to create hybrid seeds of crop plants.

9801853 Chloroplast Transformation of Monocots
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Grant 98-35300-6973; $160,000; 2 Years

Almost all commercially available genetically engineered plants have been obtained through the nuclear genome. However, genetic engineering of the chloroplast genome is an attractive alternative to the nuclear genetic engineering due to its ability to express higher levels of foreign proteins and preclude escape of those genes via pollen to wild species, creating super-weeds. Therefore, genetic engineering of the chloroplast genome is environmentally safer than nuclear genetic engineering. However, chloroplast genetic engineering has been accomplished only in tobacco so far. Recently, we developed a universal cassette that can be used to transform chloroplasts of various plant species, including monocots. This cassette has revealed almost perfect DNA sequence homology among several species including tobacco, corn, rice and soybean. We also recently developed unique chloroplast transformation and regeneration protocols for corn and rice. Preliminary analysis of rice DNA indicates that the foreign gene has been integrated into the chloroplast genome of putative transformants. We propose to establish chloroplast transformation systems for corn and rice by the following approaches: 1) construct new cassettes containing various selectable marker genes; 2) introduce cassettes into unique corn and rice targets and regenerate transgenic plants; 3) characterize transgenic plants, and 4) analyze progeny resulting from self and reciprocal crosses to study inheritance and stability of the introduced trait. These investigations should open a novel approach to genetically engineer plants that are environmentally safe.

9801148 Universal DNA chip for Genotyping Single Nucleotide Polymorphisms
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Strengthening Award; Grant 98-35300-6599; $95,000; 2 Years

We propose to develop a new DNA microarray based method to score single nucleotide polymorphisms (SNP) using the oligonucleotide ligation assay (OLA). One hundred SNP markers will be mapped using a population derived from a cross between a rapid cycling crucifer and a club-root disease resistant cabbage variety. The DNA microarray will be produced with DNA fragments that have optimal hybridization characteristics. As these fragments will be cloned in plasmids, this project will generate material resources that researchers in public sector laboratories can use for SNP genotyping in other crops.

9801298 Cloning a *Phaseolus vulgaris* Gene with a Broad Resistance to Potyviruses

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Grant 98-35300-6373; $260,000; 3 Years

Several of the plant disease resistance genes that have been cloned via positional cloning or transposon tagging were first identified as monogenic traits. These genes share sequence motifs that implicate them in signal transduction. This finding suggests that plant defense mechanisms are by no means simple. For instance, genetic and molecular analyses of the tomato-*Pseudomonas syringae* pathosystem have detected, in addition to *Pto*, other genes involved in the defense mechanism. The *Phaseolus vulgaris*-bean common mosaic virus pathosystem is complex but amenable to genetic and molecular analysis. There are five independently inherited disease resistance loci that singly or in combination produce 11 resistance spectra against an array of 15 strains of BCMV. We have selected the *I* gene for positional cloning because it confers resistance to BCMV and eight other distinct potyviruses, and it is also able to interact with 5 other resistance genes. Molecular characterization of this gene may reveal important features of the defense mechanisms against potyviruses in legumes and in other taxa. The first objective of this proposal is to construct a contiguous DNA sequence around the *I* gene with overlapping large insert clones selected from a *Phaseolus vulgaris* BAC library (available from Dr. Sally Mackenzie's lab, Purdue University). We have screened this library with a DNA marker we have found tightly linked to *I*. The second objective is to identify a genomic DNA fragment that contains the *I* gene by means of an *Agrobacterium*-mediated transient gene expression system developed for detached bean leaves.

9801451 1998 Congress on In Vitro Biology - Plant Program

Parrott, W. A.; Jones, T.
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Grant 98-35300-6199; $2,500; 1 Year

The advent of the era of genomics is presenting new challenges and opportunities to plant scientists who work in a variety of disciplines. A working definition of genomics was provided in the Editorial in the October 24, 1997, issue of Science (278:555), which stated "In the broadest sense, the issue (genomics) is concerned with taking the sequence information that is the output of the project to the next level - making it useful in studies ranging from understanding the function associated with the sequences to making the transition from gene discovery to the clinic." As applied to plant-based agriculture, the transition is not to the clinic, but to farmers' fields. It is evident that plant scientist working in a variety of disciplines, ranging from gene discovery in a laboratory to scientists engineering newly discovered genes into plants, will have to work together to achieve the final goal of letting farmers be the ultimate beneficiaries of plant genomics. To achieve that goal, this meeting will bring the different types of scientists together so that they may learn about each others' disciplines, and expose them to the latest emerging tools, such as molecular cytogenetics, which is rapidly emerging as an area that impacts on plant tissue culture and genetic engineering, as it is providing tools which can be used to address long-standing issues in somaclonal variation and expression and integration of transgenes. Accordingly, we are also planning a symposium to familiarize the SIVB Plant Program membership with this area.

9801149 Pyramiding Bt and Soybean Genes for Insect Resistance

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Grant 98-35300-6229; $175,000; 3 Years

Careful development of soybean varieties carrying insecticidal genes from *Bacillus thuringiensis* (Bt) should have a major impact in reducing losses caused by insect pests and the use of insecticides. However, such plants have two major limitations: 1) Bt alone will not control all the insect pests which attack soybean, and 2) susceptible pests are remarkably adept at developing resistance to insecticides, including Bt. It is already clear that certain management strategies should delay the development of Bt-resistant pests, but these could be more effective if combined with strategies to supplement Bt with other insect resistance genes. This is called gene stacking, and we are pursuing this strategy using a technique called marker-assisted selection (MAS) to add other soybean insect resistance genes to a Bt-soybean line. MAS uses DNA sequences located near desirable genes as "tags" that can be analyzed to screen for the presence of those genes. This powerful technique allows breeders to select plants carrying desirable combinations of genes without having to screen them for the actual trait(s). This is especially useful in our situation because: 1) Bt masks the presence of any other resistance genes, 2) plants can be screened when they are very young, and 3) DNA-based screening is not affected by environmental factors. With molecular markers, we are able to, for the first time, develop plants carrying various combinations of insect resistance, and these will be evaluated for resistance to several insect pests to determine the value of stacking these genes in elite varieties.

9801368 Physical Organization of the Mla (powdery mildew) Resistance-gene Cluster in Barley
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Grant 98-35300-6170 ; $200,000; 2 Years

Powdery mildew of grasses (barley, wheat, rye, and oats) is an excellent model system for investigating disease resistance mechanisms among fungal pathogens and their cereal hosts. Resistance to this disease in barley is conferred by genes designated *Ml*. Approximately thirty variants have been identified at the *Mla* resistance-gene cluster. Each of these variants enables the barley plant to elicit a unique reaction in response to infection by different isolates of *Erysiphe graminis f. sp. hordei*, the causal agent of powdery mildew disease. This abundance of variability makes the *Mla* locus an ideal target for genetic and molecular analysis. We have used DNA markers to identify large-insert BAC (Bacterial Artificial Chromosome) and YAC (Yeast Artificial Chromosome) clones spanning the *Mla* cluster. During the tenure of this funding period we will complete a contiguous physical map and initiate high-throughput BAC DNA sequencing of the *Mla* cluster. The primary sequence data will be used to identify and position putative *Mla* alleles via a computational approach. Mutational analysis and functional complementation via transformation will be used to confirm candidate alleles. Detailed, high-resolution maps of specific areas of the genome are necessary to investigate traits of agronomic importance. Completion of this project will provide a significant contribution towards understanding the complexity of host resistance to obligate fungal understanding the complexity of host resistance to obligate fungal pathogens. This will put us in position to identify the genetic and molecular mechanisms underlying the biological bases of plant-pathogen interactions in agriculturally important members of the *Gramineae*.

9801138 MARs and Genes in Colinear Regions of Grass Genomes; Relationships between Composition, Structure and Function
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Grant 98-35300-6167; $270,000; 3 Years

The goal of this study is to follow the relationship between plant genome organization, spatial folding and functional capacity. The method will be to analyze the structural and compositional organization of native chromosome continuums, including MARs, as structural elements, and their relationship with the environment they function in: genes, repetitive DNA, transposable elements, etc. The importance of better understanding such a relationship is felt each time when creating a transgenic plant with desired features is attempted. Phenomena such as *trans*-inactivation, position-effect, repeat-copy silencing, co-suppression, etc., are still beyond our understanding and our capacity for control. The very fact that multigene families exist and function successfully in the genome, while experimentally introduced genes are not only silenced, but often impair the expression of the homologous host genes, illustrates our lack of knowledge of some fundamental requirements that might have been neglected. Therefore, one venue of our research will be to observe, describe and analyze the composition and structure of native colinear systems. The second will be to approach the putative involvement of the structural elements, MARs (matrix-attachment regions) in their functioning. A major outcome will be a broader insight into how complex grass genomes are organized as a first step towards understanding how they function. They will create the background for new hypotheses and experimentally testable models. These will be important not only from an intellectual point of view, challenging our comprehension of genome function, but will have consequences of major practical and agronomical importance.

9801131 Complex Trait Mapping: Statistical Methodology and Significance
Deorge, R. W.
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Grant 98-35300-6173; $175,000; 3 Years

Locating the regions on chromosomes responsible for different agroeconomic or yield traits is of increasing interest to both the genomics community, as well as the general public. If regions of chromosomes are correctly identified, then these regions can be introduced into any crop, and even related species for agricultural benefits. The statistical methodologies for locating these regions rest on the assumption that the measured traits are independent, and are infinite in precision. For some traits (discrete and categorical) this is not the case, as we see only that an individual has the disease or not, or that the trait is simply the number of lesions. Additionally, many of the current methodologies for locating associated regions assume that the individuals measured are independent of one another. Often there is a pedigree structure imposed the mating design that can and should be taken into account when the date is analyzed. The statistical methodology proposed will take into account the nature of the trait that is being measured, its relationship to other related traits, and the mating structure employed. Each area of this proposal addresses the long-term goal of USDA Plant Genome in developing novel techniques for genome mapping. Millions of dollars are spent supporting research that fits into the above description. A majority of these projects collect data with little thought in which type of analysis will best serve their objectives. It is important that the money, time, and effort spent collecting data be met with correct methods of statistical analysis.

9801145 Statistical Genetics in Agriculture: An Interdisciplinary Future

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Grant 98-35300-6133; $2,000; 1 Year

The 6th Purdue Symposium on Statistics will be held June 17-23, 1998, at Purdue University, West Lafayette, Indiana. This meeting will concentrate on the new and evolving of Statistics in many fields which require interdisciplinary efforts. One portion of the meeting will be a two day (June 21-23, 1998) conference on Statistical Genetics entitled, “Statistical Genetics in Agriculture: An Interdisciplinary Future.” The integration of Genetics and Statistics into a cross-disciplinary effort supports an expanding view in Statistics. Interdisciplinary interaction focuses on a teamwork strategy, drawing together people of different training for the actual solution of national problems, as exhibited by current United States agriculture concerns. The main objective of this conference is to bring together bench scientists and applied theoreticians in agricultural genetics to discuss their current and future needs. The proposed conference supports the interdisciplinary problems currently facing the plant genomics community in the United States.

9801282 Positional Cloning and Comparative Genomics of Soybean Cyst Nematode Resistance

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Grant 98-35300-6168; $250,000; 3 Years

One of the most destructive diseases on soybean is the soybean cyst nematode (SCN). Recently, it has become clear that just a few genes control SCN resistance and one, rhg1, is especially important. We have mapped the genes for SCN resistance and determined their roles and interrelationships. Specifically, we have pinpointed rhg1 on the soybean genetic and physical maps as a starting point for positional cloning. In the process, we have characterized other interesting genes physically linked to rhg1, as well as related SCN resistance genes elsewhere in the soybean genome. In our USDA-NRICGP project, we propose to: 1) Complete the isolation and characterization of rhg1, 2) Study the genomic region around rhg1, and 3) Compare this soybean genomic region to related segments of the soybean genome as well as two model plant organisms, *Medicago truncatula* and *Arabidopsis thaliana*. In the process, we will develop a better understanding of the genome evolution of disease resistance genes and also create tools for applied plant breeders. Moreover, the positional cloning tools we create will help to lay a foundation for the wider soybean genomics community and begin to tie soybean genomics together with molecular model plant systems.

9801279 Stadler Genetics Symposium

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Grant 98-35300-6134; $10,000; 1 Year

In today’s scientific arena an inter-kingdom interaction of scientists and students is essential for rapidly advancing our knowledge on genomes. Most of the techniques used are the same (with minor variations) regardless of which kingdom in which the research is being conducted. The Stadler Genetics Symposium is intended as a Symposium designed to bring scientists to the University of Missouri campus to discuss their research which is very important for the utilization and dissemination of existing technology. Many symposiums organized in the past have suffered by splitting up the various disciplines into separate rooms and having concurrent sessions. This type of arrangement does bring the scientists together but does not allow for much interaction. The Stadler Genetics Symposium will have all the participants in the same room listening to all presentations in order to generate interactions. This type of arrangement has worked very well in the past. In addition, a Symposium organized along inter-kingdom research is critical for a better understanding of the results and what they mean in relation to genome evolution and manipulation.

9801153 Chromatin Elements, Transgene Silencing, and Position Effects

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Grant 98-35300-6231; $270,000; 3 Years

We have shown that DNA sequences called MARs (for Matrix Attachment Regions) that can hind to a protein matrix in the cell nucleus can also cause large increases in gene expression when they are attached to a gene being transferred in a genetic engineering experiment. MARs can also substantially reduce the loss of gene activity in the second generation after gene transfer, a result that may have important implications for plant breeding. The first part of our project is based on the idea that MARs reduce the likelihood of a natural “gene silencing” process that is known to inactivate foreign DNA in plant cells. We outline experiments to test this idea and learn more about how MARs are able to exert the effects we have previously observed. In the second part of the proposed work, we describe a search for other types of DNA sequences that may affect genes introduced into plant cells. In this case, our object is to find elements that will protect (‘insulate’) foreign genes from processes that condense parts of chromosomes to make them inactive. If we could find such ‘insulator’ elements, we believe we could substantially improve the efficiency and reliability of genetic engineering in plant improvement programs.

9801300 Algorithms and Programs for Mapping Quantitative Trait Loci

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Grant 98-35300-6191; $285,000; 3 Years

Mapping quantitative trait loci (QTL) is to identify the genomic location of genes affecting quantitative traits. Identifying those genes is the first step toward understanding of the genetic basis of quantitative traits and eventual manipulation of those genes for trait improvement. The goal of this project is to develop rigorous and efficient statistical algorithms and user-friendly computer programs for QTL mapping data analysis. Significant progress has been made in the last several years on developing the concept and implementing the procedures of composite interval mapping - a statistical method - in diverse populations and data structures. A computer program package called QTL CARTOGRAPHER has been developed and freely distributed to the scientific community to facilitate the general usage of the methods. Recently, we also developed multiple interval mapping to further improve the power and efficiency of mapping multiple QTL and to study epistasis of QTL. The method uses a multiple QTL model to search, fit and estimate multiple parameters including epistasis simultaneously, and thus permits the study of the genetic architecture of quantitative traits. It also offers a method for automated statistical analysis in interpreting the relationship between genotypes and phenotypes. It is proposed in this project to further develop multiple interval mapping, extend it to multiple inbred line crosses, and implement the methods in QTL CARTOGRAPHER.

9801869 Molecular Characterization of a Major Gene Cluster of Wheat

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New Investigator Award; Grant 98-35300-6130; $150,000; 3 Years

Wheat is the most important food grain crop in the world. Because of the increasing world population, food scarcity looms on the horizon. Cloning and understanding the mode of action of the useful genes will fuel the biotechnological advances in wheat production and quality. We have reported that the majority of wheat genes are present in clusters encompassing small submicroscopic chromosomal regions. I have chosen the largest gene cluster of wheat which is present on the short are of wheat homoeologous group 1 chromosomes. The gene cluster region contains at least 40 agronomically important genes and is marked by 98 DNA markers. The long term objective of the proposed study is to clone the whole gene cluster region so as to access useful genes present in the cluster. The specific objectives of the current proposal are: (i) to enrich the gene cluster region with molecular markers previously mapped in wheat and other *Triticeae* species by comparative mapping, and (ii) to generate a high resolution map for the gene cluster region by physical mapping on deletions lines and genetic linkage analysis on various *Triticeae* mapping populations. Successful completion of the project will be the first step towards cloning the entire gene cluster region which eventually make it possible to clone and characterize the useful genes present in the gene cluster.

9801135 Organization of Genes in a Small Genomic Region of Related Grass Species

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Grant 98-35300-6165; $200,000; 2 Years

Many traits in plants are encoded by gene families. The ability of seeds to act as a sink for nitrogen and amino acids during plant development is also controlled by a gene family. They encode proteins whose sole function is to store amino acids, which are called storage proteins. Many of their genes are clustered, thereby providing an entry point for studying areas of high gene density in plant genomes. Generally, spaces between genes, called intergenic regions, are not regarded as rich in genetic information. Furthermore, it has been found that large intergenic regions are filled with repetitive DNA elements. To reconstruct the pattern of alternating genic, intergenic, and repetitive DNA sequence elements in a gene-dense section, we use comparative genomics to align genomic regions from related species that differ in size: maize: 2.5 MD, sorghum: 0.7 MB; and rice: 0.4 MB. As a starting point, we use the conserved sequence element, php20725, which is linked to a cluster of 22-kDa zein genes in maize to clone genomic DNA from sorghum and rice. In sorghum, php20725- and 22-kDa zein-like sequences, also called kafirin genes, are present within the same 150 kb genomic genes. Rice differs from sorghum and maize by the absence of 22-kDa-like genes. By sequencing these genomic regions we will see how different sizes of cereal genomes, and the absence and presence of these storage proteins affect the organization of genes in a small chromosomal region.

9801165 Structural and Functional Analysis of a Region of the *Arabidopsis* Genome

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Grant 98-35300-6230; $285,000; 3 Years

The genome of *Arabidopsis thaliana* will be the first plant genome to be completely sequenced and will be finished in the next 3 years. As the genome is gene-dense and relatively simple in structure, it is likely that a very high proportion of genes will be recognizable by computer analysis. However, the function and regulation of most of these genes will remain obscure. We have developed a mutation system that takes advantage of a sensitive reporter gene to allow co-incident functional and expression analysis of individual genes. We have completed the sequence of the region immediately surrounding the *prolifera* gene on chromosome 4, and we have developed a population of plants that has a transposon inserted on average once every 1kb in this region. We will use these resources in a comprehensive survey of the function and expression of the 22 genes immediately surrounding *prolifera*. We will also isolate insertions in surrounding genes to recover mutable alleles for mosaic analysis, to investigate epigenetic effects on reporter gene expression, and to assist in positional cloning of other mutations in this region. This will be one of the first comprehensive studies of reverse genomics on a defined region in plants. This region of the genome may be closely related to similar regions in the maize and rice genomes. Because of this similarity, we hope to assess the relative value of reverse genetics in model and in crop plants.

9801159 Identification and Function of QTL Required for or Affecting Acylsugar Biosynthesis

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Grant 98-35300-6180; $140,000; 2 Years

Acylsugars confer resistance in *L. pennellii* to most of the important pests of tomato. Our prior work included studies of the acylsugar biosynthetic pathway and mapping some of the quantitative trait loci (QTL) associated with acylsugar production. Our eventual goal is to determine the function of and interactions among the major genes for acylsugar biosynthesis. The current objectives are to identify and locate the remaining QTL affecting acylsugar production, and to demonstrate that these regions, or a subset of them, are sufficient for production of the moderate or higher level of acylsugars required for insect resistance. The experiments will also provide information regarding how these QTL affect acylsugar production. A determination of the QTL required for acylsugar production is necessary if markers are to be used for transfer of these QTL, and acylsugar-mediated pest resistance, into tomato and to eliminate detrimental traits brought into the acylsugar-producing plants through linkage drag. Since tomato has the simplest and best-characterized genome in the *Solanaceae*, information obtained in tomato could be useful in the improvement of other solanaceous crops. Localization of the major QTL associated with acylsugar production in tomato is also the first step towards eventual map-based cloning and transfer of acylsugar production to non-solanaceous crops. In general terms, this project involves research to develop the knowledge and tools to engineer a metabolic pathway. Therefore, the results could also be a useful model in future modifications of other metabolic pathways.

9801274 Integration of QTL, Candidate Gene, and Comparative Sequence Analysis for Crop Improvement

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Grant 98-35300-6170; $160,000; 2 Years

Integration of genetic information from different species and methodologies can greatly enhance our understanding of plant traits by providing information about underlying genes that control the trait. This project will demonstrate the integrative application of information from different species and different kinds of genetic analyses to crop improvement. We will identify and characterize genes controlling pre-harvest sprouting (PHS) in wheat, a condition where wheat kernels germinate on the plant prior to harvest due to inadequate grain dormancy. This condition renders the grain useless for human consumption. Although much is known about the biology of grain dormancy in maize via the use of special seed-germination mutants called viviparous mutants, the genetic complexity of seed dormancy in wheat has made it difficult to study in this species. Our genetic analyses and comparisons with other species have lead us associate genes for resistance to PHS in wheat with genes controlling grain dormancy in maize, rice, and Arabidopsis. We will use this information to characterize the genes controlling grain dormancy in wheat and barley. Also, we have identified and characterized the wheat gene that is similar to the maize vivipary 1 gene. Our analysis of vivipary 1 indicates that its gene functions may be similar to those in maize, and that this gene controls both kernel color and grain dormancy in wheat. Identification of the genes controlling kernel color and dormancy in wheat, and knowledge of their DNA sequence will lend predictability to the search for novel genes that produce white wheats with resistance to PHS.

9801170 Map-based Cloning and Analysis of a Fertility Restorer Gene

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Grant 98-35300-6171; $230,000; 3 Years

Nuclear fertility restorer genes modify the action of mitochondrial genes encoding cytoplasmic male sterility (CMS). The phenomenon of CMS and fertility restoration has been applied for deliberate production of hybrid seed that will give rise to fertile plants exhibiting normal fruit and seed development. Many fertility restoration genes are known to reestablish normal pollen development by suppressing the expression of an abnormal mitochondrial gene, usually by reducing the amount of the defective gene's transcript or protein product. Nuclear genes which affect the expression of plant mitochondrial genes have not yet been cloned and sequenced. We plan to clone and sequence the nuclear DNA encoding fertility restoration from the model plant species petunia. We will use a map-based cloning approach in which molecular markers close to the gene are used to identify genomic regions which carry the gene. The sequence of the gene will be compared to existing sequences from other plant, bacterial, and animal species to gain clues to its mode of action. We will also determine whether homologous genes exist in crop species. As well as providing new information about how organelle and nuclear gene activities are coordinated, nuclear regulators of mitochondrial genes are potentially valuable for controlling the expression of novel genes that will deliberately introduced into mitochondrial genomes in the future.

9801283 Enhanced Genetic Map and Molecular Breeding Tools for Sunflower

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Grant 98-35300-6166; $285,000; 3 Years

Sunflower (*Helianthus annus L.*), is the fifth leading producer of oil and second leading producer of hybrid seed in the world and is widely grown in the U.S. for food and oil and as an ornamental. This project is geared towards developing molecular tools for increasing the speed and efficiency of commercial sunflower breeding programs and gaining an understanding of the genetics of economically important traits in sunflower. The specific objectives of this project are to (i) develop genetic markers for automated high-throughput genotyping (genetic screening), (ii) increase the density of the genetic map of sunflower, and (iii) map genes underlying economically important traits. Wild population, land races, and other exotic germplasm sources are vast storehouses of genetic diversity. This diversity has not been tapped by commercial oilseed sunflower breeders for many traits because crosses to exotic germplasm sources introduce inferior and superior genes and the former are more frequent than the latter in exotic lines. This research should (i) increase our understanding of genetic differences between exotic and elite lines and lead to a greater understanding of the genetics of several economically important traits in sunflower and, (ii) create tools for efficiently probing exotic germplasm for superior genes and introducing superior genes from exotic to elite lines. These tools can be used by plant breeders, geneticists, and the seed industry to access the genetic diversity of the sunflower family, increase hybrid seed yields, and broaden the genetic diversity of the elite gene pool, thereby decreasing genetic vulnerability.

9801365 Effects of the Fr1-Vrn1 Interval and Candidate Genes on Cold Hardiness in Wheat

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Grant 98-35300-6186; $165,000; 2 Years

Severe cold can damage and kill winter wheat seedlings. In recent years extreme cold in the Pacific Northwest and Midwest have caused million dollar losses to winter wheat crops. Winter kill losses to growers need to be reduced and a stable supply of wheat maintained for the milling, baking, and export industries. Our goal is to reduce winter kill damage by identifying DNA markers that can be reliably used to increase cold hardiness. Obtaining DNA markers linked to cold hardiness is desirable because cold hardiness is a complex trait. Present selection methods rely on field survival studies that are subject to variable winter weather conditions. Recent molecular studies that are subject to variable winter weather conditions. Recent molecular studies indicate genes that confer increased cold hardiness are located on the Fr1-Vm1 interval of wheat chromosomes 5AL and that there are just a few critical regulatory genes in plants that control cold hardiness. We plan to identify DNA markers for very cold hardy wheat genes based on linkage to the Fr-1Vm1 interval and specific cold-responsive genes control a significant amount of variation for cold hardiness and warrant manipulation by linked molecular markers in breeding programs. Results of this research will help characterize agriculturally important genes, and provide new genetic resources and reliable markers for genetic improvement of cold hardiness of wheat.

9801827 Genome Introgression for Improving Hybrid Spring Canola

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Grant 98-35300-6287; $170,000; 3 Years

Consumption of canola oil in the U.S. has risen to 1.2 billion pounds/year, but our production of the crop accounts for less than one-fifth of our consumption. Most U.S. production is limited to areas of the Midwest where Canadian spring type cultivars are well adapted. These canola cultivars are planted in the spring and harvested the same year. Winter cultivars, which are planted in the fall and must overwinter to set seed the next year, are the other major type of canola. Although winter forms are not adapted to most of the upper Midwest, they represent a novel source of genetic diversity for spring canola. We previously showed that crossing genetic diversity from winter into spring forms of canola substantially increases the sed yields of spring hybrids grown outside of the traditional canola belt. Hybrid cultivars are now commercially feasible using a genetically engineered pollination control system, and we plant to use this systems for testing genes from winter canola in spring hybrids. We previously identified two yield-enhancing genes from a winter canola cultivar, and one objective of this study is to verify the effects of these genes in the same genetic background and to test these genes in different genetic backgrounds, including a commercial hybrid combination. Another objective is to introgress and identify yield enhancing genes from two new sources. Our long term goal is to develop high yielding spring hybrids for the U.S. by combining yield enhancing genes from several genetically diverse sources into a single hybrid combination.

PLANT GENETIC MECHANISMS

Panel Manager - Dr. John B. Ohlrogge, Michigan State University
Program Director - Dr. Liang-Shiou Lin

This program area supports studies addressing the basic cellular, molecular and genetic processes which contribute new information required for the development of novel approaches to crop and forest improvement. Innovative research is emphasized in the following areas: (1) characterization of agriculturally important genes and gene products, (2) relationship between gene structure and function, (3) regulatory mechanisms of gene expression, (4) interactions between nuclear and organellar genomes, (5) mechanisms of recombination, transposition, replication, and repair (6) genetic mechanisms affecting diversity in natural and crop/forest plant populations

9801427 Dissection of EF-1A Regulation and Function in Maize Endosperm

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Grant 98-35301-6544; $100,000; 2 Years

Cereals provide 50% of the dietary protein for humans and can comprise 70% of the protein intake for people in developing countries. Unfortunately, cereals do not provide a nutritionally balanced source of protein. The most abundant proteins they contain, the prolamins, are devoid of several amino acids essential for monogastric animals; the most limiting of these is lysine. The maize *Opaque2* gene encodes a transcription factor that regulates prolamin gene expression, and its mutation pleiotropically leads to the synthesis of lysine-containing proteins. We recently found *opaque2* causes a significant increase in elongation factor-lA (EF-1A). EF-1A is lysine-rich (10%), and it accounts for 1% of the protein in the endosperm. Remarkably, the concentration of EF-1A is highly correlated ($r^2 = 0.9$) with the total lysine content of the seed. Besides being a protein synthesis factor, EF-1A binds the endoplasmic reticulum and is a component of the cytoskeleton. The goal of this research proposal is to determine basis of the relationship between EF-1A and the other major lysine-containing proteins. We will use two experimental approaches to address this question: 1) We will investigate the genetic regulation of EF-1A synthesis by determining the number of loci contributing to variation in the EF-1A levels, and 2) We will identify genes whose expression is coordinately up-regulated with EF-1A in genotypes that synthesize large amounts of EF-1A, and we will determine if the concentration of the proteins they encode is highly correlated with the lysine content of the endosperm.

9801627 Defining Photoregulation of the Blue/UV-A Light Inducible *hliA* Gene

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Grant 98-35301-6445; $210,000; 3 Years

During the middle of the day, photosynthetic organisms are exposed to very high levels of irradiation. The absorption of excess light energy can lead to the formation of highly reactive oxygen species that could destroy cell structure and function. This proposal describes work aimed at understanding how cyanobacteria perceive excess light, and will reveal some of the responses that excess light triggers and how these responses help the organism survive. Specifically, we are analyzing the expression of a gene encoding a protein called HliA, which resembles the light harvesting chlorophyll *a/b* binding proteins in plants and is part of a complex that is located in the thylakoid membranes. This complex is associated with the pigments zeaxanthin and chlorophyll a and may enable the cyanobacteria to efficiently dissipate excess absorbed light energy. The level of this complex with associated HliA increases upon exposure to excess irradiation. We are isolating mutants that cannot properly control the synthesis of HliA in order to define the molecules that sense the light environment and that transduce the light signal into chemical signals which directly control the synthesis of the HliA protein complex. Developing an understanding of the ways in which light alters the expression of genes and the synthesis of specific macromolecular complexes may ultimately enable us to use molecular tools to make plants more sensitive to changes to light levels, thereby allowing them to rapidly develop responses that help them cope with their dynamic environment.

9801371 Protein Tyrosine Phosphatases and Their Function in Higher Plants

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Grant 98-35301-6172; $110,000; 2 Years

Plants perceive their environment by a number of signaling pathways that transmit the external signals into response in plant growth and development. Understanding the molecular machinery responsible for signal transduction would provide a basis for manipulating plant growth and development to suit the need of food supply for mankind. Our long term goal is to elucidate how plants change their developmental course under various environmental stimuli.

Numerous studies have shown that protein phosphorylation and dephosphorylation at serine/threonine residues of protein molecules play a key role in signal transduction in higher plants. However, function of reversible tyrosine phosphorylation is controversial because none of the kinases or phosphatases responsible for this reaction has been identified in higher plants. Our preliminary studies identified the first tyrosine-specific protein phosphatases from a higher plant, providing a critical stepping stone for addressing the function of tyrosine phosphorylation. In particular, the expression of this phosphatase is inducible by environmental stress factors including high salt and low temperature, suggesting a possible role of this gene product in stress responses. We plan to further characterize the expression pattern of the gene and identify its *in vivo* function by a series of biochemical and molecular genetic approaches. Results from these studies will have significant impact on signal transduction and will provide information for engineering crops that respond to environmental signals in a desirable way.

9801829 Repair of UV-induced DNA Damage in *Arabidopsis*
Britt, A.B.
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Grant 98-35301-6058; $l20,000; 2 Years

Our research has two aims: the characterization of processes by which plants repair UV-induced DNA damage, and the isolation of mutants defective in repair. We use these mutants to: l) Distinguish between functionally independent repair pathways, 2) Determine the relative importance of each repair pathway, and to some extent each type of DNA damage product in the biology of UV-resistance, and 3) Provide a foundation, via map-based cloning, for the isolation of genes involved in repair.

The seedlings of *Arabidopsis thaliana* will be used as a model for the study of UV repair throughout this work. We have already developed a "root-bending" assay for UV-sensitivity and have employed this to identify mutants defective in dark repair and photo-repair. We are currently using conventional genetic techniques to map these mutations and determine how many genes are involved. We have already identified, through mutational analysis, a number of different genes required for the repair of UV-induced DNA damage. We have also identified, through computer analysis of the *Arabidopsis* genome database, a number of sequences that are very similar to genes already identified as important for repair in humans and yeast. Our major goal, during this period of funding, is to determine whether these repair-related sequences correspond to our repair-related mutations. This will enable us to determine whether repair processes in plants are similar to those in animals.

9801376 Examining the Processes That Have Affected Genetic Diversity in Maize
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Grant 98-35301-6153; $110,000; 2 Years

There has been increasing concern over the lack of genetic diversity in cultivated crops. Genetic diversity is important for two reasons. First, it is a necessary component for breeding improvement. Second, genetically homogeneous crops can be susceptible to wide-spread and economically devastating infection. Despite the obvious importance of genetic diversity, there have been surprisingly few efforts to quantify genetic diversity in crops and even fewer efforts to understand the genetic relationships between crops and their wild relatives. Our ongoing studies are designed to better understand the processes that have shaped genetic diversity in maize and its wild relatives.

We examine genetic diversity by sampling DNA sequences from individuals representing maize and three of its wild relatives. The DNA sequences will be sampled from genes on the 1st and 9th chromosomes. The DNA data will be used to address three issues. First, previous work has shown that the maize genome may be mosaic, in that the origin of genetic diversity seems to vary among genes. The data from the project will be used to further quantify the nature of this mosaic on chromosomes 1 and 9. Second, DNA sequence data will provide insight into the long-term effects of recombination in shaping genetic diversity. Finally, the data will permit inferences about the impact of domestication on genetic diversity of maize.

9801391 RNA Editing in Plant Organelles
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Grant 98-35301-6043; $100,000; 2 Years

RNA editing is a novel post-transcriptional process that is critical to the expression of plant mitochondrial and chloroplast genes. The DNA sequence of many chloroplast and virtually all plant mitochondrial genes encodes incorrect genetic information which is transcribed into RNA and subsequently corrected by RNA editing. The editing apparatus selects specific cytidine ("C") residues and converts them into uridine ("U") residues. This conversion usually results in changing the amino acid specified by that codon, and re-codes the codon to specify the evolutionarily conserved amino acid residue.

Proteins produced from unedited RNAs are typically non-functional. The mechanism of C- to -U conversion and of editing site selection are poorly understood, yet critical for the function of the energy transducing organelles in plants, the mitochondrion and the chloroplast. This research project will utilize molecular, genetic, and biochemical approaches to identify components of the editing apparatus. In addition to the importance of this proposal in understanding novel mechanisms in gene expression, RNA editing modifies genetic information, and could potentially be used to modify gene expression.

9801377 Structure and Function of the 60S Ribosomal Subunit P-protein Complex in Plants

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Grant 98-35301-6077; $100,000; 2 Years

The synthesis of proteins is an absolute requirement for plant growth. The regulation of protein synthesis at specific stages of development, such as during grain filling, and in response to environmental stimuli, is an important determinant of crop yield. Protein synthesis is carried out by the ribosome, a cell organelle with two subunits composed of about eighty proteins and four RNAs, with the assistance of a large number of proteins called translation factors. Protein synthesis occurs when a messenger RNA (mRNA) transcript is selected and decoded by the ribosome to construct a polypeptide (protein) chain. This process is regulated at the whole cell level or by the differential selection of mRNAs. Our research will focus on mechanisms that control protein synthesis after the selection of the mRNA, during the elongation phase of translation. We will examine phosphorylated proteins of the large subunit of ribosomes, the P-proteins, that form a complex involved in elongation. We have shown that ribosomes of maize roots possess four distinct forms of P-proteins, one of which is apparently unique to plants. We will estimate the amounts of these individual proteins in ribosomes during development and in response to environmental stress. We will attempt to isolate mutants with altered composition of P-proteins. We will characterize translation elongation factor-2 that interacts with the P-protein complex during elongation. This analysis should provide information of the specific role of this ribosomal structure in the regulation of protein synthesis.

9801510 Analysis of HSP101-mediated Translational Enhancement

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Grant 96-35301-3144; $150,000; 2 Years

An informed approach towards improving plants, particularly crop species, requires not only understanding the role of genes but how their expression is controlled. An integral part of this approach requires an understanding of translation and how it is regulated. We have demonstrated that the 5' leader of tobacco mosaic viral mRNA functions as a translation enhancer in plants and identified a 102 kDa (p102) host protein that binds to the subsequence within the 5' leader that is responsible for the translation enhancement. We have now identified p102 as the heat shock protein HSP101 and demonstrated that this protein is necessary to mediate the translational enhancement from Ω and does so even in yeast. This is the first identification of a protein that functions as a specific translational enhancer to be discovered in any species and may function as the basis for determining translational competitiveness in plants. We will investigate the mechanism by which HSP101 enhances translation by determining which translation initiation factor, i.e, eIF4E, eIF4G, eIF4A, eIF4B, eIF2, eIF3, or the poly(A)-binding protein, may be involved in HSP101 function using genetic mutants of these factors in yeast. We will also determine which domains of HSP101 are required for its activity as an RNA binding protein, as a translational regulator, and for thermotolerance. We will also determine whether translational enhancement conferred by Ω is dependent on the level of HSP101 expression in transgenic plants.

9801428 Organelles Containing Neutral Lipids and Unique Proteins in Seeds and Anthers

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Grant 98-35301-6037; $110,000; 2 Years

The project studies the oils and their associated proteins in seeds and flowers. Seed oils are important in human diet, animal feed, and non-food industry. In order to satisfy the expected increase in demand and to compete successfully at the international level, we need to continue and enhance efforts in research and development for the improvement of seed oil quantity and quality.

Sexual reproduction is linked directly to agricultural productivity. Manipulation of the process will bring the enhancement of fruit and seed production, the manufacture of seedless fruits, the application of male sterility to produce hybrid seeds, the orchestration of self-compatibility, and the inhibition of sexual reproduction to promote non-fruit/seed produce formation.

Both the seeds and flowers (specifically, a cell layer called tapetum which provides nutrients to the maturing pollen) contain high amounts of oils (triglycerides). The oils are associated with abundant amphipathic proteins termed oleosins in subcellular organelles called lipid bodies. This project studies the lipid bodies in seeds and anthers.

In the seed study, we will probe the mechanism of lipase action on the maize seed oil bodies during germination. The information will delineate the mechanism of oil mobilization, whose efficiency affects seed vigor and the length of the growing season, and will give us insight into the capacity and limitation of seed lipases to act on genetically engineered oils in transformed plants.

In the flower study, we will investigate the structure, function, and ontogeny of the lipid bodies in *Brassica* anther and their discharge onto the pollen surface. The findings will delineate the function of the pollen coat on the surface of the pollen during sexual reproduction.

9801816 Molecular Basis of Transgene Expression Variation

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Grant 98-35301-6091; $150,000; 2 Years

The same DNA molecule often produces highly variable levels of gene expression among transformed plant lines. This gene expression variability has been commonly attributed to a variety of factors, including variable integration sites, copy number and structural integrity of the introduced DNA. We had previously reported using site-specific recombination to insert DNA reproducibly and precisely into a previously characterized chromosome location. Upon examining the expression of independent integration events that harbor a single-copy non-rearranged DNA at the same chromosome location, we found that expression varied among independently transformed plants. It appears that nearly half of the integrated molecules do not give an expected pattern of gene expression as though the gene has been partially "silenced". For this funding period, we will investigate whether this gene silencing phenomenon is associated with changes in gene structure and function.

Specifically, we will examine the following: (1) The initiation and accumulation of mRNA transcripts. (2) The modification of the DNA and its associated histone proteins. (3) The interaction between stable and semi-stable gene alleles in the same genome, and (4) the ability of the silenced state to regain activity when plants are grown in the presence of DNA-modifying chemicals or when new plants are regenerated from cultured cells. Additionally, we seek to determine the relative expression levels among different chromosome locations. This direction of research will further our fundamental understanding of gene expression and would contribute to the development of gene transfer and gene expression technology in crop plants.

9801495 Chromatin Structure and Gene Expression in Plants

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Grant 98-35301-6083; $120,000; 2 Years

We are interested in understanding the relationships between chromatin structure and gene regulation in plants. Chromatin is the complex of DNA and protein that organizes the genome in eukaryotes and ranges in influence from sequence-specific interactions of transient proteins that bind to the regulatory region of genes, to the less precise association of proteins that manage the genome. The general hypothesis is that there are features of chromatin that play a role in gene regulation, and that all of the necessary components that characterize regulated gene activity may not be found simply by elucidating the linear sequence of nucleotides that are in promoters and finding proteins capable of binding to the promoter. The ultimate goal of this line of research is to provide a complete nuclear framework within which to view the growing amount of data on plant promoter structure and *trans*-acting factors.

We have built an expanding understanding of chromatin structure in plants as we develop new tools and approaches for investigating the subject, and some of the results obtained under previous funding have represented firsts for the field. The genes under study code for the enzyme alcohol dehydrogenase (ADH). ADH is an essential enzyme in anaerobic metabolism, a metabolic pathway utilized by plants to survive short periods of hypoxia caused by flooding. The corn Adh genes have been the most well characterized in our laboratory, and understanding the regulation of these genes contributes to our understanding of how corn plants manage hypoxic stress induced by flooding.

9801167 Characterization of Transcription Factor IIB in Plants

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Grant 98-35301-6076; $120,000; 2 Years

The overall goal is to develop a system to evaluate the contribution and roles of transcription factor IIB (TFIIB) in the process of activated transcription in plants. TFIIB plays a central role in transcription since it serves as a bridge between two major multi-protein complexes required for the expression of all genes transcribed by RNA polymerase II. It also is the target for transactivator protein interactions in their recruitment of transcription factors to the preinitiation complex. The experimental approach will entail the construction and testing of an altered specificity mutants of the TATAA binding protein (TBP) and TFIIB to create a system of analysis that is free from the interference from endogenous transcription factors. The altered specificity TBP will be able to bind to a mutated TATAA element (T<u>G</u>TAA) in the reporter gene, and the altered specificity TFIIB will be able to bind to an altered specificity TBP that contains a second mutation that normally disrupts the TFIIB-TBP interaction. This sequential-altered-specificity system will be exploited to explore the role of TFIIB in formation of the preinitiation complex and to elucidate major pathways of gene activation in plants. By establishing modes of interaction between activator proteins and general transcription factors such as TFIIB, the potential for engineering regulated gene expression can be assessed in plants.

9801509 Study of Molecular Controls in the Regulation of Cell Wall Invertase Genes in Maize.

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Grant 98-35301-6135; $100,000; 2 Years

Sucrose is both a fuel for metabolism and a signal for gene regulation in plants. Our past genetic studies have shown that the enzyme cell wall invertase plays a crucial role in the utilization of sucrose during seed development in maize. The dominant *miniature1 (Mn1)* gene of maize encodes an endosperm-specific isozyme of cell wall invertase, INCW-2. A loss of this enzyme is the causal basis for the loss of 70% of seed weight in the *mn1* seed mutant. A molecular homolog of the *Mn1* locus, the *Incw1* (Invertase cell wall 1) gene, is expressed in metabolically active sink tissues, such as cell suspension culture which is readily amenable to metabolic manipulations; and, our data show that the *Incw1* expression is significantly modulated by sugars.

Our objectives are to understand what controls the regulation of the *Incw1* gene. Several standard molecular genetic approaches are proposed for the identification and characterization of *cis* and *trans* factors that may be critical in the control of expression of the *Incw1* gene. We believe that acquisition of such basic knowledge on regulatory genes is essential for future metabolic engineering efforts to attain efficient utilization of sugars in agronomically important organs such as developing seeds, the ultimate unit of crop productivity.

9801805 Role of Aldehyde Dehydrogenase Activity in Male Fertility of Maize

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Grant 98-35301-6545; $120,000; 2 Years

Cytoplasmic male sterility (cms) is a maternally inherited inability to produce functional pollen and is a widespread phenomenon in the plant kingdom. In many cms systems, restoration of male fertility can occur in the presence of specific nuclear genes, termed restorers. Because cms and nuclear restorer systems play an important role in hybrid seed production, an understanding of the molecular mechanisms that lead to cms and fertility restoration has direct relevance to US agriculture. Using prior USDA support, a nuclear restorer of cmsT maize was cloned. This gene *(rf2)* is the only restorer cloned to date. As such, it provides a unique resource for the study of the molecular mechanisms associated with fertility restoration in cms systems. The protein produced by the *rf2* gene is an enzyme that acts on a class of chemicals termed aldehydes. As a first step in determining the specific metabolic function(s) of the RF2 protein in male fertility, the preferred aldehyde substrates of the RF2 protein will be identified by coordinated biochemical and transgenic experiments.

9801432 Transposon Excision as a Tool to Study DNA Repair in Plants

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Strengthening Award; Grant 98-35301-6740; $100,000; 2 Years

The repair of damaged DNA is a crucial process in all cells, yet remarkably little about these processes is understood in plants. Different types of DNA damage are repaired by different mechanisms and this study will focus on repair of damage resulting when DNA strands are broken. Plants, like most other organisms, carry in their DNA mobile sequences that move from site to site on the chromosomes, and each time these "transposons" move they must be cut out of the chromosome by breaking the strands of DNA. That damage is repaired leaving a very characteristic mutation behind known as a "transposon footprint". Previous work from our lab has shown that, at least for one family of transposons, the DNA repair that creates transposon footprints is a surprisingly non-random process, but one that changes slightly depending on just what the sequence of the DNA being repaired is and on what plant the damage is examined in. The rules governing these changes and the differences observed in different plant systems remain unknown. This study examines precisely what damage is caused when the *Ac/Ds* family of corn transposons moves to a new site in the DNA. Several tests of how the repair of that damage is accomplished are also carried out. Finally, experiments are proposed to identify plant genes encoding proteins that are involved in that repair.

9801498 Control of mRNA Stability in Dicotyledonous Plants

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Grant 96-35301-3157; $150,000; 2 Years

The long-term goal of this project is to investigate the pathways involved in the degradation of mRNA in an effort to elucidate the fundamental principles that govern mRNA stability in dicotyledonous plants. We are particularly interested in the mechanisms that differentiate inherently unstable mRNAs from the majority of more stable transcripts because unstable mRNAs can facilitate rapid responses to endogenous and exogenous stimuli. Under previous USDA grants, we identified two types of sequences that trigger rapid mRNA decay in plants. The first, called DST, is highly conserved in the 3' UTR of unstable Small Auxin Up RNA (*SAUR*) genes. Unlike the recognition of DST, which appears to be unique to plants, the second instability sequence we are studying in plants, the AUUUA repeat, is also known to cause mRNA instability in mammalian cells although not in yeast. Although the AUUUA mediated decay pathway is highly active in plants, the endogenous targets of this pathway are unknown. Therefore, in addition to examining the function of these elements in engineered mRNAs, in this renewal we will pursue the identification and characterization of natural mRNAs that are degraded by the AUUUA-mediated pathway in *Arabidopsis*. Another aim will be to investigate mechanistic aspects in the decay of AUUUA and DST- containing mRNAs such as the role of putative exoribonucleases in the decay of these unstable transcripts. Beyond its contribution to basic knowledge, this project should suggest solutions to practical problems resulting from the instability of some foreign mRNAs introduced into plants for crop improvement.

9801501 1998 Gordon Conference on Plant Molecular Biology

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Grant 98-35301-6047; $8,000; 1 Year

This is to support a Gordon Research Conference to be held at the New England College, Henniker, New Hampshire, on July 19-24, 1998. The theme of the conference will be Plant Biological Regulatory Mechanisms. The objective of this conference is to bring together a wide range of plant scientists who are investigating basic plant cellular processes in a variety of plant species. The meeting will explore various regulatory mechanisms controlling many aspects of plant gene expression and function, signal transduction, cell-cell interactions, and development. There will be sessions on nuclear and cytoplasmic control of gene expression, gene silencing, metabolic control, and the molecular genetics of reproductive development. These will be followed by sessions on plant-microbe interactions, plant responses to the environment, and hormonal control. Dr. Joanne Chory will be the keynote speaker of the conference, discussing light, brassinosteroids, and *Arabidopsis* development. Talks have been chosen to emphasize mechanistic processes and themes that will recur throughout the week, leading to stimulating discussions. The conference will be attended by about 135 scientists representing a broad cross-section of young and established scientists working in academic, government, and industrial laboratories.

9801438 Expression and Regulation of S-Type Cytoplasmic Male Sterility in Maize

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Grant 98-35301-6402; $100,000; 2 Years

Virtually all the corn planted by U.S. farmers is hybrid corn. The use of cytoplasmic male sterility (CMS) is a cost-effective way to produce hybrid seed. However, the major seed companies do not use CMS because of concerns about potential disease susceptibilities and the stability of the sterility phenotype. If the molecular basis of the S-type sterility were known, we expect that the use of CMS-S in hybrid seed production would increase. The goal of this proposal is to identify the sterility-associated protein in CMS maize and to analyze the action of "restorer" genes that override the sterility determinant, allowing plants to be fertile in farmer's fields. The CMS-S-associated region in mitochondrial (mt) DNA codes for two possible proteins.

We will identify which one is the CMS protein. We will investigate the action of two different nuclear restorer-of-fertility genes whose products reduce the expression of the sterility associated region by divergent mechanisms. We have found that the standard, strong restorer gene for maize CMS-S, *Rf3*, causes degradation of the S-specific RNA transcripts. We propose that the protein coded for by the "weak" *RfA* restorer alters the form of the mtDNA such that the CMS-specific transcript cannot be expressed. We propose to find out how widespread *RfA* alleles are in the maize inbreds used for hybrid production. Essential knowledge about the mechanism of CMS-S fertility restoration should result from the proposed studies, which should help breeders decide how to use this genetic resource.

9801452 Further Dissection of Signaling Pathways Leading to Disease Resistance

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Grant 98-35301-6061; $120,000; 2 Years

The long-term goal of the project is to understand the induction pathways that lead to the establishment of plant immunity to pathogens and the molecular mechanisms that determine disease resistance in plants. Systemic acquired resistance (SAR) or "immunization" in plants can be induced by a local infection and provides the plants with long lasting protection against a wide range of pathogens. We recently cloned an *Arabidopsis* gene, *NPR1*, that is a key component in controlling SAR. Our studies have shown that in addition to its role in SAR, NPR1 may also play a role in regulating resistance responses in roots in a manner distinct from SAR. Furthermore, an NPR1-independent pathway has been found to be activated in *Arabidopsis* mutants that leads to the expression of antifungal peptides and resistance to *Peronospora parasitica* infection. To broaden our understanding of the signaling network that controls disease resistance in plants, the specific objectives of the project are: (1) Determine the role of NPR1 in root-associated resistance responses. (2) Continue the epistasis analysis of the *cpr* mutants with enhanced resistance and the *npr* mutants with compromised resistance. This project will enable us to have a better understanding of the mechanisms by which plants establish resistance against infections and to explore new ways of generating disease-resistance in crop plants.

9801497 Impaired Translation and mRNA Decay

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Grant 98-35301-6060; $120,000; 2 Years

In the course of ongoing studies of light-regulated gene expression, we have observed that *(Fed-1)* transgenes containing either rare codons or premature termination (nonsense) codons produce markedly lower amounts of mRNA than the corresponding wild type constructs. Preliminary data show nonsense codons accelerate mRNA turnover, and it is likely that at least some rare codons have a similar effect. We now propose to characterize these two accelerated decay processes using *Fed-l* as a convenient, highly manipulatable, model system. The goals of this project include defining the sequence requirements for mRNA decay initiated by rare and nonsense codons. The nonsense codon effect will also be further analyzed, with particular reference to the effect of nonsense codon position on nonsense-mediated mRNA decay. Preliminary results indicate that nonsense codons in the first 3/4 of the mRNA are more effective than those near the stop codon.

Proposed experiments will determine whether this position effect reflects a requirement for specific downstream instability elements or a need for termination signals near the stop codon. The results of these studies will lead to future investigations focusing on the decay pathways used when mRNA turnover is accelerated by rare codons or premature termination as compared to normal *Fed-l* mRNA degradation. Collectively, the proposed studies will considerably extend our present rudimentary knowledge of mRNA turnover in plant cells and provide a foundation for future efforts to engineer desired degrees of mRNA stability in transgenic plants.

9801392 Transcriptional Control of a Plant DNA Synthesis Factor in Geminivirus-infected Cells

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Grant 96-35301-3177; $160,000; 2 Years

Geminiviruses are a family of DNA viruses that cause serious disease in many crops. They replicate in nuclei of differentiated plant cells using host replication machinery. Mature plant cells, which no longer divide, do not contain detectable levels of DNA polymerases or associated factors necessary for viral replication. Thus, geminiviruses must induce the requisite host proteins prior to their replication. We showed that geminivirus infection causes the accumulation of the host DNA replication factor, proliferating cell nuclear antigen (PCNA), in differentiated plant cells, and that the essential viral replication protein, AL1, is sufficient for PCNA induction. More recently, we found that PCNA RNA and promoter activity are elevated in infected tissue, demonstrating that geminivirus infection activates host transcription. Our goal is to determine the molecular rnechanisms underlying geminivirus-mediated activation of host transcription.

We have isolated and sequenced the PCNA promoter from *Nicotiana benthamiana* and showed that it is activated by geminivirus infection. We now plan to identify the DNA sequences that respond to viral infection. These studies will serve as the basis for identifying the protein factors that regulate PCNA transcription in infected versus healthy cells. We predict the factors that regulate the PCNA promoter during virus infection also control its activity during the plant cell division cycle. Hence, our experiments will provide valuable insight into plant transcriptional control mechanisms and geminivirus/plant interactions. Our studies may also lead to new resistance strategies against these important plant pathogens.

9801507 Effects of Photosynthesis on Post-transcriptional Gene Regulation

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New Investigator Award; Grant 98-35301-6513; $90,000; 2 Years

Translation of mRNA into proteins is essential for protein production. The data obtained in this study will be practically applied to improving translation and ultimately protein production in transgenic plants. Recently, we found that a subset of mRNA is translationally responsive to signals generated by photosynthesis. Photosynthetic signals have a profound effect on the translation of both pea ferredoxin (*Fed-l*) and light harvesting complex B (*Lhcb*) mRNA in tobacco. This increase in translation increases mRNA stability (and therefore accumulation) of *Fed-l* but not of *Lhcb*. We propose to identify the properties of transcripts that are responsible for the response to photosynthetic signals on translation and/or the effects of translation on mRNA stability. We have identified the pea *PetE* transcript as an additional mRNA post-transcriptionally regulated by light. Preliminary data suggest that although *PetE* does not exhibit behavior identical to either *Fed-l* or *Lhcb*, it may be subject to part of the *Fed-l* light regulation pathway. We propose to further study the light regulation of *Fed-l* and *PetE* in order to better understand properties of a transcript necessary for full *Fed-1* like regulation. Furthermore, we will screen differentially selected cDNA libraries to identify other transcripts that are subject to either *Lhcb* or *Fed-1* like post-transcriptional light regulation signaled by photosynthesis.

9801381 Mechanism of Action of a Non-canonical Plant Transcription Factor

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Grant 98-35301-6041; $130,000; 2 Years

To promote long-range improvement in U.S. agriculture, it is necessary to develop a fundamental understanding of the regulatory mechanisms which control the expression of genes in plants. Although many possible model systems could be adopted, viruses have been extensively utilized to investigate gene expression in other organisms, such as bacteria, or mammals. Geminiviruses carry out a relatively well-defined program of regulated gene expression during the infection of their plant hosts, and provide a suitable model for plant systems. The viruses are readily amenable to molecular genetic and biochemical analysis. They replicate to high levels in infected plant cells, and a single viral protein (AL2) can mediate both the activation and repression of gene expression. The elucidation of the molecular mechanisms which underlie these genetic regulatory events is the long-term goal of this research project.

The current proposal is focused on the identification of signals which are required for the activation or repression by the AL2 protein of expression from specific genes. In addition to their utility as models for the regulation of gene expression in plants, geminiviruses cause agriculturally significant plant diseases. A more thorough understanding of the mechanisms by which the AL2 protein works may also assist in the design and development of geminivirus-resistant crop varieties.

9801445 Increasing Vitamin E (-Tocopherol) Content of Vegetable Oils by Manipulating -Tocopherol Methyltransferase Levels in Transgenic Oilseeds

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Grant 98-35301-6042; $210,000; 3 Years

Vitamin E is made up of a class of lipid soluble antioxidants known as tocopherols which include , , and -tocopherols. Although all tocopherols function as antioxidants, only -tocopherol is selectively retained in the human body. Based on -tocopherol supplementation studies, it is clear that elevating -tocopherol levels in the human diet decreases the risk for cardiovascular disease and cancers and in general prevents or slows a number of degenerative disease processes. However, obtaining the recommended dosage of -tocopherol is difficult in the average American diet. Dietary -tocopherol can only be obtained from plants and the major sources of dietary -tocopherol are vegetable oils, especially soybean, rapeseed and corn. Although these oils are rich in total tocopherol content, the most biologically active form, -tocopherol, is only present as a minor component and the immediate biosynthetic precursor, -tocopherol, typically predominates. This observation suggests that the enzyme -tocopherol methyltransferase (TMT), which converts -tocopherol to -tocopherol, may be limiting in these nutritionally and economically important oilseed crops. We have recently cloned the -TMT gene from the model plant *Arabidopsis thaliana* and are modifying its expression to determine the potential for increasing the -tocopherol content of seeds. This research will provide the molecular tools necessary to increase the -tocopherol content in a wide range of agricultural crops and be directly applicable to major agronomic crops. Even a modest increase in the -tocopherol content of plant oils will significantly impact the total daily -tocopherol intake by the average individual and thus provides clear benefits for human nutrition and health in the general population.

9801807 A Structural and Transcriptional Analysis of the S-locus Region of *Brassica*

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Grant 98-35301-6072; $230,000; 3 Years

In breeding programs, pollination and fertilization processes are often manipulated for the production of hybrids that surpass other varieties in yield, tolerance to adverse environmental conditions, and uniformity under field conditions. Genetic self-incompatibility (SI), in which the female reproductive apparatus (the pistil) blocks the growth of self-related pollen while allowing the growth of genetically unrelated pollen, is a natural mechanism that promotes out-crossing and is adaptable to schemes for production of hybrid seed on a commercial scale. To make full use of genetic SI systems in agricultural applications, it is critical to understand the molecular basis of the highly specific cell-cell communication events that underlie pollen recognition in these systems. We have shown by molecular cloning technology that in the mustards (cabbages, cauliflower, broccoli, canola, and radishes), SI is under the genetic control of a complex S locus within which are embedded several genes with co-adapted functions.

Among these functions, we have identified a receptor system that operates in cells of the pistil to perceive and transduce an as-yet uncharacterized pollen-borne signal that is also expected to be encoded by the S-locus complex. Our work is aimed at elucidating the overall organization of the S locus and at identifying its genes. These studies may allow the future transfer of an SI recognition system into crops other than mustards in which natural barriers to self-fertilization do not occur.

9801441 Molecular Analysis of *Arabidopsis* Poly (A) Binding Proteins

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Grant 96-35301-3146; $100,000; 2 Years

Poly(A) binding proteins (PABPs) control two important events in gene expression in eukaryotes: mRNA turnover and translational initiation. While PABPs are essential for eukaryotic cell, we only begin to understand their multiple roles. *Arabidopsis* PABPs are encoded by a large, divergent multigene family, individual members of which are differentially expressed during development of the plant. We have found that at least 3 members of the *Arabidopsis* PABP multigene family can complement PABP-deficient mutant of yeast. Like yeast PABP, these plant PABPs could activate poly(A) shortening and translational initiation. However, they did not restore the linkage of deadenylation and decapping that normally exists in yeast. Also, *Arabidopsis* PABPs interacted *in vitro* with the *Arabidopsis* translational initiation factor, eIFiso4G. While the corresponding yeast proteins also interact, the structural basis of this interaction appears to be distinct. Thus, the divergent *Arabidopsis* PABPs demonstrate conservation of some, but not all, of the basic functions and features of eukaryotic PABPs.

The long term goal of this renewal is to elucidate the roles and molecular mechanisms of PABPs in post-transcriptional control in plants. Specific aims of this renewal are to characterize the interaction of *Arabidopsis* PABPs with the translation initiation factor, eIFiso4G, and to identify novel proteins interacting with PAB5 protein. We will also begin to address the question which step of translational initiation is stimulated by plant PABPs.

It is anticipated that the experiments proposed here will enhance our understanding of post-transcriptional control in plants. This information will be helpful in further development of methods to control and manipulate the expression of endogenous and foreign genes in plant cells.

9801810 Molecular Mechanisms of Geminivirus Replication

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Grant 96-35301-3147; $140,000; 2 Years

Geminiviruses are important pathogens of food, vegetable, and fiber crops cultivated in dry subtropical and tropical locales. A knowledge of geminivirus replication mechanisms is necessary if we are to develop an understanding of how these viruses interact with their hosts to cause disease. Once identified, key interactions may be blocked, destroying the viruses' ability to replicate in its hosts and cause disease. Thus, studying geminivirus replication positively contributes to the improvement and sustainability of U.S. agriculture.

This research concerns mechanisms and host interactions used by tomato golden mosaic virus (TGMV) to regulate its replication and gene expression programs. TGMV AL2 protein (TrAP) is a transcription factor that stimulates viral coat protein gene expression by an activation mechanism in mesophyll cells, and by derepression in vascular tissue. TrAP does not bind DNA sequences known to mediate its activity; instead, we hypothesize that a cellular protein(s) interacts with TrAP and directs it to responsive genes. Using genetic and biochemical methods, we intend to identify this host protein, as well as other cellular proteins (general transcription factors) that interact with the TrAP activation domain to stimulate transcription. We also intend to characterize a viral DNA sequence which mediates TrAP activity, and determine if competition between a host protein:TrAP complex and viral replication proteins regulates a switch from DNA replication to late gene expression. Finally, the structure of TrAP itself will be investigated so that its direct interactions with cellular proteins and viral DNA sequences can be better understood.

9801436 A Multifunctional DNA Binding Protein Required for Gene Transfer to Plants

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Grant 98-35301-3178; $100,000; 2 Years

Genetic engineering of plants depends upon the ability to introduce foreign genes (DNA) into plants in such a way that the new genes remain intact and are stably inherited. *Agrobacterium tumefaciens* is the most widely used means to insert foreign genes into plant chromosomes. *Agrobacterium* provides proteins that protect transferred DNA from rearrangements, reducing the occurrence of detrimental changes common with artificial transformation methods, which use naked DNA. VirE2, the most abundant *Agrobacterium* protein that accompanies DNA into plants, is the subject of this proposal. VirE2, a DNA-binding protein, functions in plant cells. *Agrobacterium* exports this protein into plant cells where it coats DNA also transferred from *Agrobacterium*; this protects the foreign DNA from destruction. VirE2 protein may also facilitate transport of DNA across the plant cell's nuclear membrane. Once inside the nucleus, VirE2 is believed to bring the incoming DNA into contact with plant chromosomes, thereby promoting integration of foreign DNA into plant DNA. Because VirE2 plays a crucial role in stable transfer of foreign genes into plant cells, we will examine export of VirE2 protein into plant cells and the role VirE2 plays in the nucleus. This research may help us develop a means to introduce DNA into plant chromosomes at specific locations, which would improve the reliability of genetically engineered plants.

9802199 A Viral Suppressor of Gene Silencing in Plants
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Grant 98-35301-6078; $130,000; 2 Years

We have identified a sequence from a plant virus that suppresses posttranscriptional gene silencing when expressed in tobacco. This viral suppressor of gene silencing comprises the 5' proximal region of the tobacco etch virus (TEV) RNA genome. The goal of the research proposed here is to exploit this newly discovered viral suppressor of gene silencing to help elucidate the mechanism of gene silencing in plants, and the research plan has two specific aims. The first aim is to determine the nature of the gene silencing that can be suppressed by the TEV sequence and how it compares to known classes of gene silencing. Does the viral suppressor interfere with transcriptional silencing, post-transcriptional silencing or both? Information gained from these experiments will lead to a better understanding of the link (if any) between the two major classes of gene silencing.

The second aim is to use deletion/mutation analysis to identify the minimal regions of the viral sequence required for the suppression of silencing. Gene silencing is an important genetic mechanism in plants with both theoretical and practical implications. The discovery of a viral sequence that suppresses the induction of gene silencing opens a new and promising avenue to understand the mechanisms that regulate this pathway in plants. In a practical sense, the discovery has significant potential to improve yield in technologies that use plants to express foreign gene products.

9801380 Flavanone Branch Point Regulation in Model Plants with Varied Flavonoid Profiles
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Strengthening Award; Grant 98-35301-6514; $90,000; 2 Years

Flavonoids are a group of chemicals made by plants that are important for flower color, fruit color, some fruit flavors, and protection of leaves from ultra-violet light damage. Flavonoids sometimes cause insects to avoid eating plants, and some play important roles in attracting insect pollinators. Because they are present in large amounts and since plants are a large part of the human diet, there has been a lot of research investigating effects of flavonoids on human physiology. For example, some flavonoids have anti-inflammatory properties and others can prevent tumor formation. There are nine general types of flavonoids that are made using a common "core" biosynthetic pathway. Other plant enzymes further modify these compounds giving each its specific chemical character. The variety introduced by the modifying enzymes results in over 5000 different flavonoid compounds identified so far. No single type of plant makes all of these; rather, there is a specific pattern to the flavonoids made by any one species.

This research is designed to characterize two enzymes, one "core" enzyme, and one "modifying" enzyme, in an early branch point of the flavonoid biosynthetic pathway and eventually to elucidate the biochemical, physiological and molecular control of this branch point. The research will advance our understanding of how much and what specific kinds of flavonoids different plants make. Once understood, it may be possible to develop plant varieties with altered abilities to produce flavonoids such as insect feeding deterrents, medicinal compounds, desirable flavor and color components, or undesirable compounds.

9801821 The Mechanism of Cap-independent Translation in Plants
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Grant 98-35301-6084; $100,000; 2 Years

A goal of this laboratory is to understand how plants make proteins (protein synthesis) from a messenger RNA template. Most cellular messenger RNAs contain a functional group (m7GpppG) called a "cap" at their 5' end. The function of the cap is to assist in the initiation of protein synthesis and protect the messenger RNA from degradation until it is used to program protein synthesis. There are several viral messenger RNAs that have evolved mechanisms for protein synthesis that do not require the presence of the cap for highly efficient initiation of protein synthesis. One of these, satellite tobacco necrosis virus (STNV), is our model messenger RNA to study this unusual mechanism. We will determine what features of STNV RNA allow this message to initiate protein synthesis independent of the cap. These features may be sequence dependent, structure dependent, or both. We have identified an initiation factor that participates in the initiation at the 5' untranslated region of normal cellular messenger RNAs that binds to a specific region of the STNV RNA 3' untranslated region. In addition, we will explore the possibility that normal plant cellular messenger RNAs may also use some type of cap-independent initiation of protein synthesis during certain times of development or stress.

It is important to understand the mechanism of cap-independent translation to further our knowledge of how plants make proteins. This information is necessary to facilitate the engineering of plants to make proteins of higher nutritive quality and to produce proteins of economic or medicinal value in plants.

9801496 Epigenetic Interactions of the Maize *R-Stippled* Gene Complex

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Grant 98-35301-6059; $110,000; 2 Years

This award uses genetic and molecular methods to study *R-stippled* (*R-st*), an allele of the maize *red color* (*r1*) locus involved in a naturally occurring form of copy number- dependent gene silencing. *R-st* is comprised of four tandem copies of the *r1* gene. It is able to heritably reduce expression of certain other *r1* alleles on homologous chromosomes, a phenomenon referred to as paramutation. Additionally, three of the four *r1* genes silence each other in a copy number-dependent manner. The objectives of this proposal are to complete the molecular characterization of all four *r1* genes and their promoters, assay cytosine methylation patterns and expression of the *r1* genes in *R-st* and selected truncation derivatives and to initiate genetic work aimed at testing the minimal requirements for the ability to induce paramutation and the effects of *R-st* on other *r1* genes located on the same chromosome. This work will test the role of a family of transposable genetic elements shared between genes in *R-st* and all tested *r1* alleles sensitive to paramutation in silencing and whether changes in *r1* gene number and phenotype are correlated with changes in cytosine methylation and steady state mRNA levels. This work will contribute to a better understanding of copy number-dependent gene silencing in plants and may be useful in developing methods to overcome this phenomenon following introduction of genes into many plants by gene transfer methods integral to many current efforts in crop improvement.

9801502 Role of Wound-inducible Polygalacturonase in Plant Leaves

Ryan, C.A.
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Grant 98-35301-6046; $120,000; 3 Years

We have discovered a new subfamily of plant cell wall degrading enzymes, called polygalacturonases, in wounded tomato leaves. This class of enzymes had never been found in leaves of any plant and it is synthesized in leaves as one of several defensive proteins in response to insect and pathogen attacks. The wound-inducible enzyme can produce cell wall fragments at wound sites that activate defense genes to help with wound healing and defense against pathogens. The mRNA has been isolated and both the mRNA and enzyme have been shown to be induced in leaves in response to an a powerful 18 amino acid polypeptide wound signal, called systemin, released at sites of herbivore attacks. In response to systemin the plants become resistant to attacking insects. This indicates that the initial signal systemin produces an enzyme that releases secondary signals (cell wall fragments) that can activate defenses against pathogens. This indicates that systemin has a much broader signaling role in plants than previously known. The understanding of the response could lead to new approaches to engineer crop plants so that they perceive pest attacks very early and mount a faster and stronger defense response against insects and pathogens.

9801443 Molecular Characterization of Genes That Influence Abscission and Cell Separation

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Grant 98-35301-6764; $146,502; 2 Years

Abscission is a developmental process regulating detachment of organs from the main body of the plant. Abscission occurs in a specialized band of cells and involves changes in the cell walls that lead to the separation of abscission zone cells at the middle lamellae. This important biological mechanism protects plants from disease, environmental stress, insects and other predators, as well as provides a method for efficiently discarding organs that are no longer needed. The abscission process is of economic importance in that the timing of leaf, flower, and especially fruit abscission has an enormous impact on yield and quality of a number of crop species. Additional forms of cell separation are also important to agriculture and forestry: for example fruit ripening and paper making. A more complete understanding of the regulatory systems in plants that control the timing and progression of cell separation processes will lead to the development of biotechnological methods for improving the performance and quality of a variety of agricultural crops.

Our basic approach is to identify and characterize the genes that control cell separation processes in plants using two model systems that we have developed in *Arabidopsis*. Floral organs abscise shortly after anthesis and we are using this system to isolate mutants which disrupt the timing of the abscission process. The second system involves the identification of mutations that cause cells in *Arabidopsis* seedlings to undergo cell separation at specific stages in development. These genes are being cloned and molecular and biochemical investigations are underway.

PLANT GROWTH AND DEVELOPMENT

Panel Manager - Dr. Donald E. Fosket, University of California, Irvine
Program Director - Dr. Liang-Shiou Lin

This program area supports research aimed at increasing our basic understanding of mechanisms underlying the regulation of plant growth and development in order to achieve optimal productivity of agriculturally important crop plants and forest species. Research areas emphasized by the program include: (1) mechanisms of cell division, expansion and differentiation, (2) development and organization of meristems, (3) photomorphogenesis, (4) cell biology, including studies on cytoskeleton, membrane transport, protein trafficking, and cell wall structure and properties, (5) metabolism related to growth and development, (6) hormonal regulation of growth and development, (7) signal transduction mechanisms related to growth and development, and (8) analysis and control of growth patterns.

9801270 Molecular Genetic Analysis of Potassium Nutrition in Plants
Zhu, J. K.
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Grant 98-35304-3797; $105,000; 2 Years

Potassium nutrition is essential for plant growth and a key factor controlling crop productivity. Potassium uptake by plant roots is mediated through multiple transporters. The activities of potassium transporters are regulated by environmental factors such as potassium availability and sodium stress. The goal of this project is to improve our understanding of the regulation of root potassium transport. Specifically, we will carry out genetic and physical mapping of the *sos2* mutation at high resolution with the eventual goal of cloning the *SOS2* gene from the model plant *Arabidopsis thaliana*. The *SOS2* gene is critical for potassium nutrition because mutations in *SOS2* render the plant unable to grow on culture media with low levels of potassium or with high levels of sodium. Cloning of *SOS2* will enhance our ability to develop rational strategies for increased plant potassium efficiency as well as decrease the reliance on potassium fertilizers.

9801767 Structure and Function of Chloroplast Signal Recognition Particle
Carnegie Institution of Washington; Department of Plant Biology; Stanford; CA 94305
Grant 98-35304-3703; $105,000; 2 Years

Recently we discovered that chloroplasts contain a novel protein complex, chloroplast signal recognition particle (cpSRP), that plays a role in the insertion of proteins into the thylakoid membrane. As the proper insertion of membrane proteins into the thylakoid membrane is essential for efficient photosynthesis, understanding the details of this process has important implications for agriculture. The substrates for cpSRP include the light harvesting chlorophyll proteins (LHCP). These proteins provide a useful model for study because the insertion of LHCP into the membrane can be reconstituted *in vitro* using chloroplast lysates. The principal goal of the proposed research is to determine the structure of cpSRP and elucidate its role in LHCP trafficking. We have determined that cpSRP is a tetramer comprised of two proteins, cpSRP54 and cpSRP43. One objective is to define the parts of the two proteins that interact and to measure binding affinities. A second objective is to similarly define how cpSRP interacts with the substrate LHCP. Although we have demonstrated a requirement for cpSRP, we also found that an additional soluble factor is necessary for insertion of LHCP into the thylakoid membrane. A third objective is to identify this factor using biochemical and recombinant DNA techniques and address how the factor mediates LHCP insertion in concert with cpSRP. The successful implementation of this grant will result in the reconstitution of LHCP transport using purified soluble components and consequently will facilitate detailed biochemical studies on the insertion of LHCP into the thylakoid membrane.

9801266 Aminocyclopropanecarboxylate Synthase: Reaction Mechanism of Suicide Inhibition
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Postdoctoral Fellowship; Grant 98-35304-6743; $90,000; 2 Years

Ethylene is a plant hormone that stimulates seed germination, leaf and flower senescence, and fruit ripening. The enzyme that catalyzes the committed and rate-limiting step of ethylene biosynthesis in plants is 1-aminocyclopropane-1-carboxylate (ACC) synthase, which catalyzes the conversion of S-adenosylmethionine (SAM) to ACC. ACC is then converted to ethylene by another enzyme, ACC oxidase. Because ACC synthase performs such a central role in ethylene biosynthesis, it is a logical target for control over plant growth and development. In fact, the natural regulation of ethylene biosynthesis in plants is in part effected by irreversible inactivation of ACC synthase by SAM; approxi-mately one in 30,000 interactions between SAM and ACC synthase results in the inactivation of the enzyme.

The objective of the proposed studies is to elucidate the chemical reaction mechanism of inhibition of ACC synthase by SAM. Deuterium exchange experiments will be utilized in order to differentiate between three proposed mechanisms for inactivation, and deuterium and tritium kinetic isotope effects will be measured in order to determine the nature of the rate-limiting step(s) in the inactivation reaction. Elucidation of the mechanism of inhibition of ACC synthase by SAM will provide information that could be useful in the design of specific and effective chemical inhibitors of ACC synthase. These compounds could inhibit ethylene production in plants, thus allowing exogenous control over plant growth, development, and senescence.

9801409 Function of Aux/IAA Proteins in Auxin Signaling
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Grant 98-35304-6713; $160,000; 2 Years

The plant hormone auxin is essential for plant form and function. It has been implicated in regulating a myriad of developmental and cellular processes by altering basic patterns of gene expression. Thus, the understanding of auxin signal transduction pathways leading to gene expression raises the prospect of improving plant performance in numerous beneficial ways. Early-inducible genes provide a viable paradigm to dissect auxin signaling. Three major classes of primary auxin-responsive genes have been isolated, among which the *Aux/lAA* gene family has emerged as a model to study early gene activation and early gene function in auxin action. *Aux/lAA* genes encode short-lived nuclear proteins with properties reminiscent of transcription factors. Aux/IAA proteins interact with Auxin Response Factors (ARFs) via a C-terminal domain, which is conserved in both protein families.

ARFs bind to auxin responsive DNA elements of early auxin-inducible genes and may regulate, in concert with Aux/ IAA proteins and other factors, secondary gene expression in response to auxin. Elucidation of Aux/IAA protein function by molecular and biochemical approaches is the goal of this proposal. Aux/IAA proteins have affinity for DNA, and we will employ complementary *in vitro* and *in vivo* selection methods to identify and characterize DNA binding sites. Isolation of potential target genes by genetic selection in yeast and by subtractive cloning procedures in *Arabidopsis* will be initiated. In a second strategy to elucidate the role of Aux/IAA proteins in auxin-regulated gene expression, we will use a modified two-hybrid interaction screen in yeast to identify interacting, accessory proteins.

9801353 Function and Expression of the Ovule Development Gene *INO*
Gasser, C.S.
University of California, Davis; Section of Molecular and Cellular Biology; Davis, CA 95616
Grant 96-35304-3707; $105,000; 2 Years

Ovules of higher plants are the direct progenitors of seeds. Because seeds are the primary means of propagation of annual crops, and are the major source of nutrition for humans and farm animals, ovule development is an essential component of crop productivity. We have identified and cloned an *Arabidopsis* gene, *INNER NO OUTER* (*INO*), which is essential for normal ovule development. Mutations in the *INO* gene cause the outer of the two integuments (precursors to the seed coat) to initiate in an incorrect location and prevent further development of this structure. Seeds which develop from these ovules lack the outer layers of the seed coat and exhibit defects in stability, dormancy and germination.

We have shown that *INO* encodes a protein with properties of a DNA-binding transcriptional regulator. We will use the cloned *INO* gene as a tool to study the biochemical properties of the encoded protein, to identify and characterize the sequences responsible for regulating the expression pattern of *INO*, and to study effects of other mutations on the *INO* gene expression. Completion of the proposed research will significantly contribute to understanding molecular processes regulating ovule and seed development, and will provide new tools for manipulation of these processes for crop improvement through genetic engineering.

9801768 Phytochrome Chromophore Biosynthesis and Its Regulation in Transgenic Plants
Lagarias, J.C.
University of California, Davis; Section of Molecular and Cellular Biology; Davis CA 95616
Grant 98-35304-6404; $240,000; 3 Years

Phytochrome is a light receptor that enables plants to optimally utilize sunlight from the environment. Like the pigments in our eyes, phytochrome functions to detect light and to modify plant growth and development accordingly. We are interested in how phytochrome is assembled from its individual components, a protein and a pigment (i.e., chromophore). The overall goal of these investigations is the development of new methods to alter yield- reducing responses of plants to the natural light environment through modification of the synthesis and assembly of a functional phytochrome chromophore. We have recently developed a promising approach to regulate light-mediated plant development by expression of a pigment degrading enzyme from mammals in transgenic plants. The feasibility of this approach has been established in two model plant systems and we ultimately hope to extend these studies to other plants species. The ability to selectively modify the levels of phytochrome in agronomically important crop plants has profound implications for agriculture. For example, it is desirable to inhibit the phytochrome response in crop plants whose growth is adversely affected by neighboring vegetation. Compounds which alter the synthesis of the phytochrome pigment could also be used to selectively inhibit the germination of undesirable weed species and/or to alter the photoperiod sensitivity of crop plants. Photoperiod insensitive varieties of important crops will enable formation of new hybrid varieties with extended geographic range and potentially with improved resistance to disease, drought and other environmental stresses.

9801840 Regulation of Compound Leaf Development in Tomato

Sinha, N.
University of California, Davis; Section of Plant Biology; Davis, CA 95616
Grant 98-35304-6679; $100,000; 2 Years

The shoot apical meristem of a plant produces leaves in succession over its life time. Leaves are the primary organs capable of photosynthesis and are critical to plant survival. Higher plants can produce either simple or compound leaves. Compound leaves can improve heat transfer efficiency and may help reduce herbivore damage and increase photosynthetic efficiency. The primary goal of this study will be to analyze of how a compound leaf is formed. Tomato has a compound leaf and genetic mutations exist that alter the compound leaf. These mutations have been classified into categories depending on the type of defect in leaf development that they produce. Mutations that specifically make the leaf either more or less compound than normal will be utilized to understand leaf development in tomato. Our preliminary data indicate that the tomato compound leaf may acquire its complex nature over a period of time. We will undertake developmental analyses of specific mutations to determine if the various leaf domains are specified progressively. We will also determine the role of specific genes like homeobox genes in the generation of a compound leaf. The events during leaf initiation and very early leaf development in the normal and mutant leaves will be characterized using these tools. Results from our analyses should eventually provide tools for the manipulation of leaf architecture for better plant productivity.

9801839 Component of the Thylakoid Translocation Machinery

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Grant 98-35304-6746; $105,000; 2 Years

Thylakoids are membrane vesicles located within chloroplasts, the subcellular organelles in plant cells responsible for photosynthesis. Many of the thylakoid proteins are encoded by DNA in the cell nucleus, and must be imported from the cytoplasm after synthesis. The work performed in connection with this proposal will investigate the mechanism of import of a specific subset of these proteins.

Proteins are transported across the thylakoid membrane by one of two distinct pathways. One of these is rather unusual in that the energy required for protein transport is delivered in the form of a pH gradient across the membrane by the action of light. Until recently it was thought that this pathway was unique in the biosphere. However, it is now apparent that this same pathway is present in bacteria and represents a previously unrecognized prokaryotic protein transport system.

Two mutants exist in which the delivery of proteins across the thylakoid membrane by this delta pH-dependent pathway are impaired. The proteins targeted by these mutations have been identified, and antibodies against them have been raised. We will use these antibodies as specific probes with which to follow the isolation of a complex involved in protein transport on this pathway. To this end, proteins in the thylakoid membrane will be crosslinked, and then the membrane will be solubilized with detergents. Solubilization of the desired protein complexes will then be followed using these antibodies. In this manner we will isolate polypeptides of the protein transport machinery, enabling us to start their molecular characterization.

9801425 The Role of Expansin Expression in the Organogenesis of a Parasitic Plant Haustorium

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Postdoctoral Fellowship; Grant 98-35304-6673; $90,000; 2 Years

Parasitic plants are devastating agricultural pests worldwide. I study *Triphysaria*, which is included in the same family of parasitic weeds as the witchweed species. Witchweeds are known to attack all the important cereal crops and can completely decimate entire fields. The common feature to all parasitic plants is the haustorium, the organ that serves to identify, attach, invade, and eventually parasitize the host plant. The haustorium, visualized as a swollen region covered by epidermal hairs, is formed near the root tip in response to chemical signals given off by host roots.

All plant cells are surrounded by a rigid box called the cell wall. In order for plant cells to grow, this box must be loosened to allow for cell expansion. Expansins refer to a group of proteins that aid in the cell wall loosening that occurs during plant growth. Since the formation of the haustorium requires cell growth and expansion, I will test the hypothesis that one or more of the genes encoding the expansin proteins will be activated. Furthermore, I will determine if different expansin genes are required for the root swelling and epidermal hair proliferation or if the activity of one expansin gene can fulfill both roles. The successful completion of this project will 1) determine whether expansins have a role in the formation of the parasitic plant haustorium, 2) indicate the extent by which individual expansin genes participate in different growth events, and 3) possibly indicate potential molecular targets to combat these serious agricultural pests.

9801763 Structural Characterization of Pectate Lyases and Their Plant Homologues

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Grant 98-35304-6745; 180,000; 3 Years

Pectate lyases are virulence factors that degrade the alpha-1,4-polygalacturonate (PGA) component of plant cell walls, initiating soft-rotting diseases in plants. The enzymes also liberate oligosaccharides from higher plant cell walls that function as elicitors of active plant defense reactions. The objective of the present proposal is to elucidate how interactions with plant cell wall-degrading enzymes affect the conformation of PGA and pectin fragments. Attempts by other investigators to elucidate the structure of PGA have resulted in simplified models, a 2_1 helix at dilute concentrations and a 3_1 helix in gels, but neither describes PGA under conditions present in the plant cell wall. The structure of a complex between a PelC mutant, R218K and a PGA fragment, (Gal*p*A4), has recently been solved by x-ray diffraction methods to 2.2 Å-resolution. The substrate contains two saccharide units related by a 2_1 helix and two, by a 3_1 helix. An analysis suggests that the 3_1 helix is favored.

The structure also reveals additional Ca^{2+} sites not present in the uncomplexed PelC structure. Ca^{2+} ions coordinate to protein as well as to the PGA fragment, but the linking pattern differs significantly from that suggested by the popular "egg-box" model of the plant cell wall. The present proposal will utilize the PelC R218K mutant to elucidate the conformation of longer PGA fragments, with 10 or more saccharide units. The structure of a fungal plant virulence factor, pectin lyase B (PLB), has also been solved to 1.8 Å-resolution. Complexes of the PLB R236K mutant with pectin fragments will also be determined by x-ray diffraction methods. The research should result in a better understanding of components and interactions within the plant cell wall.

9801458 Financial Support for 20th Annual Symposium in Plant Physiology

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Grant 98-35304-6921; $5,000; 1 Year

This award provides partial support for "Cell and Developmental Biology of Arabinogalactan-Proteins," the 20th Annual Symposium in Plant Physiology at the University of California, Riverside, to be held on January 21-23, 1999. The organizers are Eugene Nothnagel, Department of Botany and Plant Sciences at UCR, and Adrienne Clarke and Antony Bacic, Plant Cell Biology Research Centre, University of Melbourne, Australia.

Arabinogalactan-proteins (AGPs) are a class of structurally-complex macromolecules that are present at the surface of cells throughout plants. Typically consisting of about 90% carbohydrate and 10% protein, AGPs are the focus of increasing research interest from the viewpoints of both function in the plant and practical application in industry. Recent research points to roles of AGPs in reproductive development, vegetative development, somatic embryogenesis, and the underlying processes of cell division and expansion. Other work focuses on AGPs as major components in gum arabic, a plant gum with unique viscosity and emulsifying properties that have led to many uses in the food and other industries. *Acacia senegal* trees yielding the best gum arabic grow in the Sudan, which has produced as much as 60,000 tons of gum arabic per year at a value of $360 million. Political problems in the growing regions and quality variations due to environmental fluctuations have led to interest in developing reliable, domestic alternatives to gum arabic. The symposium will bring together scientists representing the full spectrum of AGP research and will lead to a coherent volume that reports advances in cell and developmental biology of AGPs and their practical applications.

9801359 Molecular Basis of High-affinity Potassium Transport in Higher Plants

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Grant 98-35304-6684; $100,000; 2 Years

Potassium is one of the major macronutrients in higher plants required for plant growth and development. Potassium (K^+) transport is important for many physiological processes including root and shoot growth, tropisms, cell expansion, enzyme activities, salinity stress and osmoregulation. K^+ accumulation from soils into roots and translocation throughout the plant are mediated by K^+ transporters. Genes that encode putative molecular mechanisms for K^+ transport have been isolated. By expression cloning, functional analysis in plant cell cultures, yeast, *Xenopus* oocytes and *E. coli* several gene families have been identified, which may contribute to high-affinity K^+ uptake into plant cells. At least three gene families have been proposed to contribute to K^+ uptake into plant cells including K^+ channels, the wheat *HKT1* gene and the recently identified family of *AtKUP* transporters (also named *ATKT* or *HAK*). The long-term goal of this proposal is to gain insight into the physiological roles of cloned K^+ transporter genes while focusing on a member of the *AtKUP* family. Effects of mutation of the K^+ transporter on root and shoot growth phenotypes and sensitivity to toxic cations will be analyzed.

Functional properties of *HKT1* will also be further analyzed in heterologous systems and in plants. Results from these studies will lead to a molecular physiological understanding of K^+ transport in plants and may contribute to development of future strategies for engineering improved K^+ nutrition, growth and K^+ uptake-related stress tolerance in crop plants.

9801776 Genetic and Biochemical Approaches to Understanding Cytokinin Regulation

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Strengthening Award; Grant 98-35304-6672; $90,000; 2 Years

One group of plant hormones, the cytokinins, influence many aspects of plant growth including cell division, leaf development and flowering. All plants must be able to regulate the active cytokinin molecules within tissues and cells in order to have normal development throughout each stage of the life cycle. This regulation of cytokinin is partially achieved through reversible conjugation of cytokinins to sugar molecules. Despite the central importance of cytokinin regulation to plant growth, the process is poorly understood at the genetic and biochemical levels.

We have selected a genetic mutant of the model plant *Arabidopsis thaliana* which appears to have abnormal cytokinin regulation and we believe that the *cym* (cytokinin metabolism) mutant lacks the activity of an enzyme affecting both cytokinin and adenine conjugation. Specifically, our studies indicate that the activity of a 5' nucleotidase or adenosine nucleosidase enzyme may be absent in *cym* plants. The abnormal appearance of *cym* seedlings further suggests that cytokinin levels during early seedling development are disturbed by the mutation.

This research project will fully describe the *cym* mutant and the location of the *cym* gene in the *Arabidopsis* genome. We will determine the role of the *cym* gene product in cytokinin regulation, adenine regulation and plant growth. The *cym* mutant provides a unique and powerful tool to identify an enzyme and the corresponding gene involved in the hormonal control of plant growth. As a result of this work, it may be possible to modify the growth of transgenic crop plants by altering the natural hormone levels within the plant.

9801354 Recessive Mutations Identifying Early Steps in Maize Leaf Pattern Formation

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Grant 98-35304-3732; $120,000; 2 Years

The differentiation of the basic patterns of leaf architecture and function is easily distinguished along all three axes of the maize leaf--proximodistal, dorsiventral, and lateral--making this system a powerful one for the analysis of pattern formation. The *roughsheath2* (*rs2*), *leafbladeless1* (*lbl1*), and *midribless1* (*mrl1*) recessive mutations identify genes likely to have roles in leaf pattern formation. The *Rs2* gene encodes a *Myb*-domain protein with a leaf primordium expression pattern consistent with a role in the regulation of homeodomain proteins. The *Lbl1* gene has a role in the lateral recruitment of founder cells at leaf initiation. Mutations at the *Mrl1* gene condition a delay in midvein initiation that has dramatic effects on leaf morphology. We will determine the roles of the *Lbl1*, *Rs2*, and *Mrl1* genes in maize leaf pattern formation, with the following aims: 1) To characterize the action of *Rs2*, including its mRNA and protein distribution through leaf initiation and development, its interactions with other proteins, and its potential DNA binding sites; 2) To characterize the action of *Lbl1*, including the nature of the encoded product, its pattern of accumulation during development, the cell-autonomy of its action in the leaf, and its genetic interaction with other morphological genes; 3) To characterize the action of *Mrl1*, including the nature of the product, its distribution through development, the cell-autonomy of its action, and a reexamination of the midrib as a clonal compartment.

9801346 Modulation of Ethylene Sensitivity in Tomato

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Grant 98-35304-1302; $180,000; 3 Years

Many important aspects of plant growth and development are controlled by the gaseous hormone, ethylene. The best known example is the control of fruit ripening in plants such as tomato. Ethylene is a controlling factor in the ripening process. It is clear that plants closely regulate both the synthesis and perception of ethylene during fruit development. While a great deal of effort has been directed at mechanisms regulating ethylene synthesis, very little is known about how the plant perceives the hormone.

The work to be performed here will focus on how the tomato plant measures and responds to ethylene. Tomato undergoes a number of developmentally regulated changes in ethylene sensitivity. Among these are flower abscission, fruit ripening, and senescence. We have cloned five genes encoding tomato ethylene receptors. The proteins are quite different structurally and are transcriptionally regulated in very different ways. We will use transgenic plants as well as *in vitro* protein:protein interactions to define specific roles for each of the receptors. We will determine whether altering the expression patterns of these proteins in transgenic plants can change the sensitivity of the plant to ethylene. The outcome of this work should be to define specific roles for each receptor during growth and development of the tomato plant.

9801339 Biosynthesis of Plant Cell Wall Pectic Polysaccharides

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Grant 98-35304-6772; $115,000; 2 Years

The goal of the proposed research is to understand how the major and core pectic polysaccharide, homogalacturonan (HGA), is synthesized. Pectin is a complex polysaccharide in the primary cell walls of plants that contributes to cell wall strength, ion exchange, and sieving properties and is thought to play critical roles in cell-cell adhesion and cell-cell communication. Oligosaccharides released from pectin induce plant defense responses and regulate plant development. Pectin is a food fiber that contributes to the roughage of fruits and vegetables, is an economically important gelling agent added to foods, and has beneficial effects on human health including the lowering of blood cholesterol levels, the lowering of serum glucose levels in diabetics, and the potential inhibition of prostate cancer metastasis. The goals of the proposed research are to purify and study PGA-GalAT (polygalacturonate 4- -galacturonosyltransferase), the enzyme responsible for the synthesis of the pectic polysaccharide HGA. GalAT will be purified by a combination of affinity, ion exchange and size exclusion chromatography and purified GalAT will be characterized and its amino acid sequence determined. The proposed studies will provide the biochemical information necessary to identify the gene for GalAT and will lead to the production of transgenic plants with altered pectin structure. Such transgenic plants will be useful to directly test hypotheses regarding pectin structure, pectin biosynthesis, and the function of pectin in plants and in human health. Plants producing modified pectin could serve as agricultural sources for pectin with improved gelling properties and additional beneficial effects on human health.

9801247 The Phytochrome B Subfamily

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Grant 98-35304-6677; $100,000; 2 Years

Phytochromes are key photoreceptors controlling many agriculturally important responses of plants to their light environment. These responses include seed germination, rate of stem elongation, time to flowering and the quantity of photosynthetically produced carbohydrate stored in harvested organs such as tubers and fruits. In flowering plants, phytochrome genes can be grouped into four subfamilies: *A, B, C/F* and *E*. The phytochrome B subfamily is of special interest for two reasons. Firstly, members of this subfamily control at least in part the agriculturally important responses already mentioned. Secondly, there is strong evidence that productive duplications within this subfamily have occurred in recent evolutionary time in at least three lineages of flowering plants. Our primary objective here is to determine the basis for the differential functions of two members of the B family in tomato (phyB 1 and phyB2). That is, we will determine whether differences between the two arise from different expression patterns in the plant, as opposed to the two phytochromes having inherently different biological activities. We will do this by preparing artificial minigenes and introducing them into already available mutants of both tomato phyB 1 and phyB2. As resources permit we will also begin experiments designed to test directly the hypothesis that neither of the *Arabidopsis B* phy (phyB and phyD) is a functional equivalent of either tomato phyB1 or phyB2. Improved understanding of this phytochrome subfamily will play a key role in achieving the potential of improving agricultural productivity through transgenic manipulation of these photoreceptors.

9801198 Structure and Function of the Chloroplast Stromal Processing Peptidase

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Grant 98-35304-6744; $100,000; 2 Years

The chloroplast plays the special role in the biosphere of converting light energy to ATP and reducing power, resulting in carbon fixation---a process called photosynthesis. The long term goal of this project is to understand how the photosynthetic chloroplast is assembled during plant development. Most of the chloroplast's proteins must be transported into the organelle during its biogenesis. These proteins contain specific targeting "signals" that specifically direct them in the cell to the correct compartment. Typically, this targeting signal is also essential for protein transport across the membranes of the chloroplast, but, ultimately, in order that biologically functional proteins are produced inside, it is critical that the transit peptide is efficiently removed. We have identified the protease enzyme of the chloroplast that is responsible for this essential reaction. We propose to investigate how it carries out its role, selectively recognizing its protein substrates and removing the targeting signal. It is predicted that the protease contains specific information in its structure for this reaction. This includes a metal-binding site that is highly conserved in a family of metal-dependent proteases that have evolved to cleave different substrates. In addition, the protein-protein interactions between the protease and its substrates will be examined. These studies will employ mutational analysis of the structure of the protease.

9801201 Cell Adhesion Interactions During Reproduction in Flowering Plants

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Postdoctoral Fellowship; Grant 98-35304-6682; $90,000; 2 Years

A critical part of plant reproduction begins when a pollen grain lands on and adheres to a female cell; it culminates in the formation of seed. Important crops that rely on seed production include: grains (such as wheat and rye), fruits (citrus), spices, coffee, and vegetable oils (soybean and safflower oils). Successful seed production requires a series of interactions between male (pollen) and female reproductive cells. Cell-cell adhesion is necessary throughout reproduction for efficient seed set, yet little is known of the molecules that mediate these interactions. I am investigating the molecules responsible for the initial adhesion between pollen and female cells using the flowering plant, *Arabidopsis thaliana*, a model genetic organism. Using a genetic approach, I will identify mutants with reduced adhesion between male and female reproductive cells. By mapping and cloning the mutant gene(s) responsible for reduced adhesion, I will identify gene products critical for efficient cell-cell interactions. Many benefits to agriculture and industry could be realized. These initial adhesion events may be responsible for maintaining species specificity; understanding this barrier would allow us to strengthen or weaken this boundary to the farmer's advantage. Adhesion between the pollen and female cells in plants like *Arabidopsis* is unlike that in animals because it occurs in a dry environment, thus plants provide a molecular model for dry adhesion which may have broad industrial applications.

9801838 Characterization of Herbicide-metabolizing P450s

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Grant 98-35304-6683; $105,000; 2 Years

The overall goal of this research is to elucidate molecular mechanisms activating herbicide metabolism by cytochrome P450 monooxygenases (P450s) in maize. P450 monooxygenases play significant roles in the primary detoxification of herbicides, pesticides and environmental pollutants as well as the biosynthesis of plant defense compounds, hormones, pigments and cell wall constituents. Through a variety of biochemical and molecular approaches, we will further characterize three P450s that are expressed in maize seedlings in response to naphthalic anhydride (a plant safener) and triasulfuron (a sulfonylurea herbicide). Expression of these P450s in yeast and transgenic plants will determine the extent to which CYP71C3v2, a P450 expressed in young (2.5-day-old) seedlings, confers herbicide tolerance/resistance on heterologous plants and the range of exogenous and endogenous substrates metabolized by this P450. Similar characterization in yeast will identify potential substrates for CYP92A1 and CYP95A1, two P450s expressed in older (6.5-day-old) seedlings. Characterization of the promoter for CYP71C3v2 by transgenic promoter analysis and DNA mobility shift analysis will provide the first structural analysis of a plant safener- and herbicide-inducible promoter and begin to elucidate the pathways regulating expression of herbicide-metabolizing P450s by these types of xenobiotics and environmental conditions (pathogen attack, UV light, wounding, etc.).

Overall, these data will further our understanding of the diverse, but extremely important, plant P450 gene family and factors influencing its expression.

9801261 Roles of VirD2 and VirE2 Proteins in T-DNA Nuclear Targeting

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Grant 98-35304-6675; $180,000; 3 Years

Agrobacterium tumefaciens transfers DNA (the T-DNA) to plant cells and, as such, is currently used as the major vector for bringing new genes into plants. When the T-DNA enters plant cells, it must first target to the nucleus before the T-DNA is inserted into the plant chromosomes. Two proteins, VirD2 and VirE2, associate with the T-DNA during the transfer process. Each protein contains nuclear localization sequences (NLS) that presumably are responsible for targeting the T-DNA to the nucleus. In this proposal, we shall investigate the relative roles of these two proteins in the nuclear targeting process. We shall do this by microinjecting fluorescently labeled "T-complexes", composed of T-DNA molecules associated with various combinations of wild-type and mutant VirD2 and VirE2 proteins, into plant cells. We shall monitor the rate and extent of nuclear targeting of these molecules. In addition, we shall investigate the importance of phosphorylation of amino acids near the NLS in the nuclear targeting process. An understanding of the T-DNA nuclear targeting process will help us improve the genetic manipulation of important crop plants.

9801357 Function of TOUSLED Protein Kinase in *Arabidopsis* Development

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Grant 98-35304-6676; $100,000; 2 Years

The proposed research utilizes both genetic and biochemical approaches to study the fundamental cellular processes involved during plant development. The TOUSLED (TSL) protein is an enzyme which is required for proper development in the plant *Arabidopsis thaliana*. The genes for highly similar proteins are present in *Brassica napus* (used for rapeseed oil production) and corn, and related genes are found in animals as well. The TSL protein is found in the nuclei of plant cells. The TSL protein is an enzyme called a protein kinase, and its function is to transfer phosphate molecules to other unknown target proteins in response to developmental cues.

The proposed research will explore the mechanism of action of the TSL protein in the model plant, *Arabidopsis thaliana*. This will include investigating which cells in the plant produce TSL protein. Also, plants lacking a functional TSL gene have developmental defects including abnormal flowers with reduced numbers of floral organs. In addition, the fruit structure in the abnormal flowers is disrupted. The research will include experiments to study the cellular structure in these abnormal flowers to better understand the cause of the defects. The research will also attempt to identify the target proteins which are modified by the *TSL* enzyme. Also included will be an analysis of how the TSL protein itself is modified in the cell. Studying the *TSL* gene in the model plant system of *Arabidopsis* should shed light on its function in crop plants, including corn and rapeseed.

9801400 Cytokinesis: Calcium, Calmodulin and the Cytoskeleton

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Grant 98-35304-6747; $115,000; 2 Years

Cytokinesis is the process by which a mother cell divides its cytoplasm into two daughter cells. In higher plants this event, which includes the elaboration of a new cell wall, involves the formation and coordinated activity of the phragmoplast. This structure is a complex of cytoskeletal and membrane elements that arises in the mitotic spindle interzone during late anaphase, and directs the formation *de novo* of the division wall. Previous research has defined many of the structural features of the phragmoplast and of the sequential transformation of the cytoskeleton during cytokinesis. We have also learned that calcium ions (Ca^{2+}) contribute to regulation, but details are unknown. These matters are addressed herein. We will seek information on the distribution of cytosolic and ER-bound Ca^{2+}, using the newly introduced cameleon protein, which will be transfected into BY-2 culture cells. The cameleon protein is a complex of calmodulin (CaM), M-13 (a CaM binding protein), and two different Green Fluorescent Proteins (GFPs). Upon binding Ca^{2+} this complex exhibits a change in its fluorescence emission spectrum, referred to as fluorescence resonance energy transfer (FRET). We will probe where CaM is bound to its response elements using fluorescence redistribution after photobleaching (FRAP). We will affect the activity of Ca^{2+}-CaM using peptides that exhibit high affinity for the complex. In particular we will employ the CaM binding domain of the kinesin-like calmodulin binding protein (KCBP), a microtubule motor protein that has recently been discovered in plants. Finally, we will explore the function and location of KCBP itself using specific antibodies Three different cell types will be used including *Tradescantia* stamen hair cells, *Nicotiana* BY-2 culture cells, and *Arabidopsis* suspension culture cells. A central thrust of this proposal is the study of the living cell as an intact unit. Through the introduction of select probes, coupled with video microscopy and quantitative fluorescence microscopy, we will attempt to unravel the underlying processes of cytokinesis.

9801420 *Arabidopsis* Ethylene Signal Transduction: New Interacting Components

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Grant 98-35304-6795; $240,000; 3 Years

Ethylene affects many important aspects of plant growth and development. The overall goal of our research is to understand how the ethylene signal is transmitted within the plant cell leading to physiological responses. In *Arabidopsis*, there are at least five ethylene receptors, which have sequence similarity to histidine protein kinases. A separate protein kinase, called CTR1, acts downstream of the receptors in the ethylene signaling pathway in *Arabidopsis*. The signaling mechanisms/components leading from ethylene perception (by the receptors) to the regulation of CTR1 are unknown. To address this problem, we have identified a number of different proteins on the basis of physical interaction with the ethylene receptors and/or CTR1. One objective of our research is to determine whether any of these components are indeed involved in transmitting the ethylene signal. To assist in this objective, we are developing a functional assay to identify components that can regulate the CTR1 kinase. A separate objective is to further characterize the direct physical interactions that we recently demonstrated between the ethylene receptors and the regulatory region of CTR1. By identifying new components in the ethylene signaling pathway, and by increasing our understanding of the receptor-CTR1 interactions, this work should lead to new strategies for manipulating ethylene responses for the benefit of agriculture and the environment.

9801344 Molecular Analyses of *LEUNIG*, A Regulator of *Arabidopsis* Flower and Ovule Development

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Grant 98-35304-6714; $109,000; 2 Years

The long term objective of this research is to understand the genetic and molecular mechanisms governing higher plant development. Using the flowering weed, *Arabidopsis thaliana* as a model system, we will isolate and characterize a key regulatory gene named *LEUNIG* using a map-based cloning method. Based on previous genetic analyses, mutations in *LEUNIG* cause abnormal flowers. These mutant flowers make carpels in place of sepals and stamens in place of petals. This floral abnormality was traced to a defect in the expression of another gene named *AGAMOUS*. This suggests that *LEUNIG* regulates where *AGAMOUS* is expressed.

In addition, *leunig* mutant plants have a reduced fertility. This reduced fertility is partly due to an abnormal development of the ovules, which are the crucial organ for egg cell development. To reveal the molecular nature of the *LEUNIG* gene product, we will isolate the DNA that encodes LEUNIG and subsequently determine the DNA sequences of this gene. We will also examine in which tissues *LEUNIG* RNA and proteins are made and how *LEUNIG* regulates *AGAMOUS* expression in flowers. Results obtained from these studies will lead to a better understanding of the control mechanism for region-specific gene expression. Since flowers and ovules are organs for plant reproduction, and their products (fruits and seeds) constitute a large portion of foods consumed by humans, our research may provide useful information for future genetic manipulation of reproductive organ development in agriculture.

9801335 Role of Oxylipins in the Regulation of Plant Defense

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New Investigator Award; Grant 98-35304-6508; $90,000; 2 Years

Plants use a variety of defense strategies to protect themselves from microbial pathogens and insect pests. Recent studies indicate that oxygenated fatty acids (oxylipins) play a critical role in many of these defense reactions. Some oxylipins function directly as anti-microbial agents while others, such as the plant hormone jasmonic acid (JA), serve to regulate the expression of defense genes. At present, a major gap in our understanding of oxylipins as defense agents is the molecular mechanisms by which their production is regulated. The focus of the proposed research is to test the hypothesis that oxylipin production is regulated by allene oxide synthase (AOS), the enzyme catalyzing a committed step in JA biosynthesis. Using tomato (*Lycopersicon esculentum*) as a model system, we will investigate the developmental and spatial pattern of AOS expression in healthy plants. We will also assess the extent to which AOS expression is controlled by signals generated during pest attack. Finally, a collection of tomato mutants defective in defense responses will be tested for deficiencies or alterations in oxylipin metabolism. These experiments will clarify the role of AOS and oxylipin signals in both healthy and diseased plants. The knowledge gained from this research will increase our understanding of oxylipins as natural regulators of plant defense, and may find application in the development of novel and environmentally-safe methods of pest control.

9801407 Identification of Auxin Signal Transduction Mutants in *Arabidopsis*

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New Investigator Award; Grant 98-35304-6674; $90,000; 2 Years

The plant hormone auxin is involved in many aspects of plant growth and development, yet little is known about exactly how this hormone causes changes within plant cells. We do know that when plant cells are exposed to auxin, certain genes are very rapidly switched on. An unknown number of molecules are involved in recognizing auxin after it enters the plant cell and causing these genes to be switched on. The goal of this project is to identify some of the molecules involved in this "auxin signal transduction" process. The author has made transgenic *Arabidopsis* plants that carry an antibiotic resistance gene controlled by auxin responsive promoter sequences. These transgenic plants were mutagenized, then mutant seeds were germinated on agar plates containing the antibiotic. Non-mutant plants are killed by the antibiotic, because they do not contain enough auxin to switch on the resistance gene. However, a few mutant plants were able to grow, because the resistance gene is expressed more highly in them than in non-mutant plants. One hypothesis is that these mutant lines contain alterations in the molecules involved in responding to auxin. The author proposes to test this hypothesis, by analyzing the mutant plants in detail, and cloning one of the mutant genes. For this project, the author will focus on five mutations that map to Chromosome 5. Optimal growth and development are essential for optimal and sustainable productivity in agriculture. The work described in this proposal will contribute to our understanding of the many aspects of growth and development that are controlled by auxin.

9801338 Components Regulating Thylakoid Protein Translocation

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Grant 98-35304-6771; $105,000; 2 Years

In photosynthetic organisms, the chloroplast organelle is the site of energy harvesting and generation, and this process is completely dependent upon proteins found with membranes. These proteins must be assembled into their site of action. However, while the translocation of proteins into and across membranes is an essential process for all organisms, the apparatus responsible for this process in the chloroplast is not characterized. Proteins needing assembly in the chloroplast have an essential signal sequence. Mutations within the signal sequence of cytochrome f (cytf) inhibit *Chlamydomonas* thylakoid membrane protein translocation and render cells non-photosynthetic. Genetic suppressors of the mutant signal sequences identify other proteins and their genes that interact with the signal sequence, and thus describe components that are required for membrane protein assembly. We identified six nuclear loci named tip1 through 6 for thylakoid insertion protein. The tip mutations likely identify components of a general thylakoid protein translocation apparatus. This proposal will characterize one tip gene and its product, tip2.

9801242 Regulation of the Gibberellin Biosynthetic Genes *GA4* and *GA4H* in *Arabidopsis*

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Grant 98-35304-6433; $180,000; 3 Years

Bioactive gibberellins (GAs) are plant growth regulators, which control such diverse processes as germination, stem elongation, and flower and fruit development. The final biosynthetic step to produce bioactive GAs is catalyzed by GA 3 -hydroxylase, which was previously shown to be encoded by two homologous genes, *GA4* and *GA4H*, in *Arabidopsis*. This proposal describes experiments designed to investigate the relative physiological function of these two genes in regulating the synthesis of bioactive GAs in germinating seeds, young seedlings and adult plants. This knowledge will be important to improve crops by manipulating GA levels in specific tissues to control their growth.

9801263 Acyl Modification of a Calcium-dependent Protein Kinase

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Strengthening Award; 98-35304-6510; $90,000; 2 Years

Calcium is well-known as an essential element for plant growth and plays a crucial role m information transfer within plant cells by its involvement in many basic physiological and developmental processes. Calcium-dependent protein kinases (CDPKs) are enzymes found only in members of the plant and protist kingdoms and have been proposed to mediate calcium signal transduction pathways in response to signals such as hormones or stresses such as drought. Enzymes involved in early steps of signal transduction pathways often are associated with the plasma membrane (at least transiently). CDPKs are not integral membrane proteins but many members of the *Arabidopsis thaliana* CDPK family contain amino-terminal sequences that could serve as potential attachment sites for acyl residues, such as myristate or palmitate. We have demonstrated that at least one *Arabidopsis* CDPK does have such a modification. The purpose of this work is to identify and confirm the site(s) of acylation and to investigate whether acylation is required for the biological function of this enzyme. Results from these studies will augment current knowledge of calcium-regulated processes in plant cells.

9801207 Specification and Function of Cells in the Megagametophyte of A*rabidopsis thaliana*

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Grant 98-35304-6412; $120,000; 2 Years

Sexual reproduction produces genetic variation among the offspring, a feature that has been widely exploited in plant breeding and agriculture. The formation of the embryo sac or megagametophyte is a key step in plant reproduction. The embryo sac harbors seven cells including the egg and central cell which upon fertilization give rise to the seed. Despite its importance for agriculture our understanding of the genetic and molecular mechanisms underlying this process is very limited. To identify genes specifically expressed during female reproductive development we are using a novel powerful approach, enhancer detection. Genes expressed in distinct cell types of the embryo sac are likely to play important roles during sexual reproduction. We will perform a limited analysis of genes active in these cells to gain a broader understanding of female reproduction in the model plant *Arabidopsis thaliana*. We will concentrate on the molecular characterization of a gene expressed in the egg cell. This analysis will provide us with new tools to investigate the interactions between cells in the megagametophyte. It is not known how these cells acquire their identity but it is likely that cellular communication plays an important role. To test this hypothesis we will kill the egg cell using molecular genetic methods and investigate the effect of an absence of the egg cell on neighboring cells. We will also use these molecular tools to specifically express growth regulators in the egg to probe its potential for autonomous embryo formation, a valuable trait for crop improvement and seed production.

9801361 Sixth FASEB summer conference on Mechanisms in Plant Development

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Grant 98-35304-6323; $10,000; 1 Year

The sixth FASEB conference on plant molecular biology will be entitled "Mechanisms in Plant Development" and will be held from August 15-20, 1998 at Saxton's River, Vermont. Plant development has become an enormously popular field, and the last few years have seen major discoveries that have illuminated botanical theories debated in the past. Well-characterized genetic models such as pea and snapdragon, and especially maize and *Arabidopsis thaliana*, have provided insights into the molecules behind many key processes in plant development. Rapid progress in genome mapping, sequencing and functional analysis make the isolation of mutants in virtually any aspect of plant development, and the molecular isolation of the corresponding genes, a routine procedure. We are now in a position to address mechanisms of development using these sophisticated tools. The scientific sessions in this meeting have a more mechanistic bias than previous conferences, but the overall progress through the plant life cycle, from gametogenesis to flowering, has been retained. We have added a session on epigenetics, an area of considerable biotechnological interest, and increased the emphasis on cell-cell interactions, hormonal and environmental regulation of development. The invited speakers include both young and established investigators from within and outside the U.S. Additional speakers will be drawn from attendees on the basis of submitted abstracts By bringing together investigators who study these various systems, we hope to facilitate an exchange of ideas and information on the fundamental aspects of plant growth and development that underlie agricultural productivity of crop plants.

9801334 Nuclear Import of Nucleic Acid-protein Complexes in Plants

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Grant 98-35304-6680; $105,000, 2 Years

Traffic of nucleic acid-protein complexes into the cell nucleus is a basic process which, nevertheless, has not been studied in plants. To start filling this gap, we will examine nuclear import of DNA-protein complexes (T-complexes) derived from *Agrobacterium*, a soil pathogen known to infect and genetically alter plants by transferring a segment of its DNA into the host cell nucleus. This study will help to understand and control the infection process and increase nuclear uptake of the DNA of interest in genetic engineering experiments. Also, since pathogens, such as *Agrobacterium*, often adapt existing cellular machinery for their own needs, our work will contribute to the understanding of plant nuclear import in general. The proposed research will seek to identify and isolate plant proteins that recognize *Agrobacterium* T-complexes and directly mediate their nuclear import. The isolated proteins will be analyzed and used to clone their encoding genes.

9801355 Intercellular Protein Trafficking and Leaf Development in Tobacco

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Grant 98-35304-6509; $90,000; 2 Years

At early developmental stages, a plant leaf is incapable of photosynthesis and is a sink for photoassimilates. As the leaf matures further, it becomes photosynthetically active and a source of photoassimilates. This sink-to-source transition is irreversible and has profound impact on the physiology and productivity of a plant. Plasmodesmata, the intercellular connections in plants that allow transport of molecules between cells, change functions and perhaps also structures in certain veins of a tobacco leaf to contribute to this transition. Where and how plasmodesmata change their functions remain poorly understood. We have recently used a viral protein, the cucumber mosaic virus movement protein (CMV 3a MP), that can be transported intercellularly to study the function of plasmodesmata in tobacco leaf development. We fused a green fluorescent protein (GFP) from jellyfish to the CMV 3a MP so that the trafficking functions of 3a MP:GFP fusion protein can be monitored under a fluorescence microscope. In this funded project, we will use CMV 3a MP:GFP fusion protein, produced in a transgenic tobacco plant, to monitor changes in the transport functions of plasmodesmata at specific cellular boundaries in a sink-to-source transition leaf. Electron microscopy will be used to investigate the ultrastructure of plasmodesmata with changes in function. The project is expected to provide fresh insight into the functions of plasmodesmata in plant leaf sink-to-source transition. The information should be useful in future work to engineer crops with modified intercellular transport functions to improve productivity and resistance to pathogens.

9801780 *In Vivo* Assay of Cell Wall Modifying Enzymes in Expanding Cotton Cotyledons

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Grant 98-35304-6779; $210,000; 3 Years

Much of the growth of plants is through expansion of cells. Cells in plants have a lower concentration of water inside them compared to the almost pure water surrounding the cells in a well watered plant because of the presence of various solutes. Thermodynamics dictates that water will move to a region of lower water concentration unless there is a force to prevent it from doing so. Thus, the cells will have a natural tendency to expand. Uncontrolled expansion would lead to bursting of the cells. It is the cell wall that restricts the movement of water into the cells because of its resistance to stretching. Since every plant cell is surrounded by a cell wall, plant growth is heavily dependent on an increase in cell wall area. Our goal is to find out which enzymes are involved in modifying the cell walls during expansion.

We have devised a general assay system to quantitate the level of almost any cell wall modifying enzyme within the extracellular spaces of expanding cotyledons using fluorescent labeled purified oligosaccharides derived from cell wall polysaccharides, a specific single oligosaccharide for each enzyme. The assay involves injecting the fluorescent substrate into the living plant, allowing the enzyme time to act, rinsing the products out of the tissue, and then detecting them by capillary electrophoresis. It is very specific and sensitive. We will use the assay to determine which hydrolytic enzyme activities are correlated with cell expansion.

9801260 Rho Family GTPases in Plant Development: A Genetic and Cell Biological Analysis

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New Investigator Award; Grant 98-35304-6670; $90,000; 2 Years

Understanding how plant organs (e.g., pollen, seeds and embryos) grow can contribute to programs that seek to alter those organs in beneficial ways. A key component in the formation of all plant organs (morphogenesis) is the spatial coordination of the cell growth that produces those structures. The spatial pattern in which a plant cell divides or expands is influenced externally by signals from neighboring cells and/or its environment, and internally by the cytoskeleton (the protein filament networks that define the cell's architecture). Members of the Rho GTPase family of proteins serve as molecular switches in cell signaling during morphogenesis. In several well-characterized eukaryotic organisms (yeast, *Drosophila*, mammals), Rho GTPases can coordinate several cellular processes, including the organization of the cytoskeleton, the formation of new cell wall, and the progression of the cell division cycle.

Using the model crop *Zea mays* (corn), we will investigate the functions of two Rho family proteins, ROP1 and ROP6, during development. We will address whether the cellular distribution of the ROP proteins provides cues that orient the expansion or division of plant cells. Furthermore, we will characterize plants that contain transposon-induced mutations in the genes that produce the ROP proteins, to determine whether alterations in these genes affect plant growth. Understanding how these important molecules influence plant cells may lead to novel genetic approaches for manipulating plant growth.

9801398 Genes Encoding Cytokinin Metabolic Enzymes

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Grant 98-35304-6511; $90,000; 2 Years

Cytokinins are plant hormones which mediate cell division and growth. In order for normal development to occur, the level of this group of hormone must be precisely regulated. Presumably, biosynthesis, metabolism and perception are possible routes of regulation. However, the mechanism of such controls are not well understood in plants. The project centers on the metabolism of zeatin, a naturally occurring and the most abundant cytokinin, in beans. The long term goal is to identify genes encoding zeatin metabolic enzymes and determine how these genes are turned on/off. During this grant period, a specific enzyme and gene controlling the formation of *O*-xylosylzeatin are being studied. Specifically, the processing of the enzyme and its subcellular location will be determined. In addition, transgenic plants already generated, overexpressing the enzyme will be analyzed. As zeatin is known to affect seed/fruit development and *O*-xylosylzeatin transferase is highly expressed in immature bean seeds, modification of the enzyme level via altered gene expression is likely to improve grain/seed yield of cereals and legumes.

9801258 Molecular/Electrophysiological Analysis of G-protein Regulation of the Guard Cell Inward K^+ Channel

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Grant 98-35304-6681; $105,000; 2 Years

During photosynthesis, the green mesophyll cells in plant leaves use the sun's energy to incorporate carbon dioxide into carbohydrates and to produce oxygen. Both photosynthetically fixed carbohydrates and oxygen are essential to life on Earth, including human life. Carbon dioxide enters the leaves through microscopic pores called stomata, and water vapor is lost from leaves through these same pores. Regulation of pore aperture, which is performed by cells called guard cells, is therefore very important so that plants are neither starved of carbon dioxide nor desiccated by excessive water loss. Guard cell control of stomatal apertures involves the flux of ions, particularly K^+, across the guard cell membrane. Research presented in this proposal is focused on elucidating the role of a class of signal-transducing proteins, the "G-proteins," in the control of transmembrane K^+ movement. We will focus on the potential role of a new G-protein subunit that we have cloned, called AtXLG. We will manipulate levels of active AtXLG protein in single guard cells and assess the impact on K^+ fluxes. This research will provide information on the guard cell mechanisms used by plants to balance the opposing priorities of maximizing carbon dioxide uptake and photosynthesis while minimizing water loss. Results may elucidate guard cell signal transduction pathways that are potential targets for breeding to improve plant productivity or drought tolerance.

9801269 Orientation Mechanisms of the Cortical Microtubule Array in Higher Plants

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Grant 98-35304-6668; $120,000; 2 Years

Plants are comprised of millions of cells. Plant cells are unique, partly because they possess cell walls, which physically confines each cell. This rigid wall, in growing cells, is composed primarily of cellulose, the most abundant (and the most economically important) polymer on Earth. Plants do not deposit cellulose in a haphazard manner, rather its deposition is highly ordered. Experimentally manipulated plants, which have aberrant ordering of cellulose, appear grossly abnormal. This grant contains experiments designed to provide insight into how plant cells affect the normal deposition of cellulose. During plant cell growth, long cellular elements, termed microtubules, are found in the cortical region of the cell where they interact with the plasma membrane (the membrane separating the cytoplasm from the rigid cell wall). The cellulose synthesizing complex resides on, and spans the width of, the plasma membrane; this arrangement allows the complex to interact with the cytoplasm, while depositing cellulose on the outside of the plasma membrane. By a mechanism that is not fully understood, cortical microtubules serve as guiding rails which direct the movement of the cell's cellulose synthesizing machinery. Thus, when cortical microtubules are highly aligned, likewise are cellulose fibers. Experiments are proposed to test the idea that the deposition of cellulose affects the alignment of cortical microtubules which, in turn, affect the deposition of cellulose. In other words, we will determine if cortical microtubules and cellulose comprise a self-rectifying system which work together to insure that cellulose is deposited correctly during normal cell growth.

9801408 ABA Signal Transduction by Phospholipase D

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Grant 98-37304-6669; $105,000; 2 Years

The germination of seeds is controlled by plant hormones. In barley, the hormone gibberellin promotes germination whereas abscisic acid inhibits this process. The broad goal of this research is to characterize the molecules that translate the abscisic acid signal to the regulation of the cellular activities in the seed that prevent germination. Abscisic acid activates an enzyme, phospholipase-D, that then triggers the abscisic acid signaling pathway in the cell. This proposal seeks to define whether this activation occurs at the cell membrane or at some internal site within the cell. The second goal is to define what other cellular factors are required to couple activation of the abscisic acid receptor to increased activity of the phospholipase-D. The final goal of the research is to clone the phospholipase-D gene for this abscisic acid activated enzyme. The structure of the gene will then be used to help understand how the abscisic acid brings about activation of the enzyme and how the structure of phospholipase-D might be manipulated to alter abscisic acid action. By understanding how such specific molecules are involved in the hormone systems that promote or inhibit seed germination, it should be possible to develop strategies to manipulate grain quality or alleviate major seed associated crop losses such as pre-harvest sprouting in cereals.

9800473 Gordon Research Conference on the Plant and Fungal Cytoskeleton

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Gordon Research Conference; University of Rhode Island; PO Box 984; West Kingston, RI 02892-0984
Grant 98-35304-6678; $6,000; 1 Year

A Gordon Research Conference on the Plant and Fungal Cytoskeleton will be held August 9-13, 1998 in Andover, New Hampshire. The cytoskeleton is intricately involved in growth and development as a result of its function in such activities as mitosis, cytokinesis, polarity determination, organelle placement, and directed cell expansion. A better understanding of the plant and fungal cytoskeleton is therefore critical to efforts aimed at improvements in crop productivity and control of fungal pathogens. The conference brings together active researchers in the field to explain their recent results, engage in discussion, and plan future collaborative projects and the sharing of biological profiles. The conference organizers widely surveyed the research communication for suggestions regarding the most exciting work being done currently, and in setting up the program of speakers, they aimed to achieve a balance between presentations on plants and fungi.

Emphasis in the program is placed on biological processes such as signal transduction, cytokinesis, cell-cell communication and polarity determination. In addition, several speakers will present research that makes use innovative techniques in real-time analysis of dynamic cellular processes, genetics and molecular manipulation. The program includes speakers from Japan, the United Kingdom, Switzerland, Australia, Germany and the United States. Whenever possible, attempts were made to select junior level investigators to make presentations. In a further attempt to encourage participation by junior members of the scientific community, much of the external funding obtained to support the conference will be used to defray expenses of students and postdocs.

9801419 Cellular Functions of Calmodulin-related TCH3 Protein

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Grant 98-35304-6715; $115,000; 2 Years

Plants are capable of perceiving stimuli in their environment, including harsh winds and temperature fluctuations. To survive in diverse and fluctuating environments, plants have evolved the ability to alter their physiology such that they are adapted to their particular environment. For example, plants exposed to heavy winds will develop stems and branches with increased mechanical stability; plants subjected to cold will undergo changes that make them more freezing resistant.

Knowledge is limited concerning the exact nature and mechanisms of these physiological alterations that plants undergo in response to environmental stress. Proteins compose the machinery which enable cells, and hence, organisms to react to stimuli. This work is focused on one protein, called TCH3, which likely plays a critical role in the processes by which cells sense and respond to environmental stimuli. TCH3 is rapidly and strongly turned on in response to diverse environmental stresses, including temperature extremes and harsh winds.

In addition, TCH3 protein is capable of binding calcium ions and thus may mediate cellular responses to calcium ion regulation in cells. We aim to determine how TCH3 works and the consequences of its action on individual cells and plants. Elucidation of this fundamental problem in plant biology may lead to the ability to manipulate the environmental stress-induced processes such that one could activate advantageous responses even in the absence of an inducing stimulus, and, conversely, inhibit disadvantageous responses in the presence of environmental stress. Such manipulations may lead to enhanced plant growth and production under diverse environmental conditions.

9801401 Defining *Arabidopsis* Xylem and Phloem Protease Substrates Using Phage Display

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Grant 98-35304-6507; $93,000; 2 Years

Cell function and identity are, in part, determined by the combined action and properties of proteins in the cell at a given time. The complement and activities of proteins are determined by the sum effect of protein synthesis plus modifications made to existing proteins. Protein degradation is the ultimate modification and is a controlled process that serves to remove proteins from cells. Protein degradation, therefore, is a mechanism for regulating cellular identity and function. Taking advantage of the ability of *Arabidopsis*, a model genetic system for higher plant research, to undergo secondary vascular development in roots, cloning, characterization and localization of xylem and phloem proteases will be conducted. These experiments will serve as the foundation for the future goal of defining the physiological substrates of vascular proteases. Information obtained as a result of the proposed experiments is expected to advance our understanding of the role of intracellular proteolysis in regulating the function and identity of cells in vascular tissues that are vital to numerous aspects of food and wood production including water and mineral transport, cellulose biosynthesis and sucrose transport. In addition, some cell types under investigation are engaged in programmed cell death which is a poorly understood yet important component of numerous aspects of plant reproduction and vegetative growth. It is possible, therefore, that information gained from these studies will allow the development of bioengineering strategies for regulating various properties of vascular tissues and for regulating the many processes that depend on programmed cell death.

9801259 Cytosolic and Membrane Proteins in Cell-plate Biogenesis

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New Investigator Award; Grant 98-35304-6671; $100,000; 2 Years

Dividing plant cells are separated by the formation of a new membrane-bound compartment known as the cell-plate. Assembly of this unique organelle is initiated in mitosis when Golgi-derived secretory vesicles begin to fuse with one another across the equatorial plane of the cell. As additional vesicles fuse, the cell-plate grows outward until it joins with the original cell plasma membrane, bisecting the two daughter cells. Despite the importance of this process for plant morphogenesis, little is known about the underlying molecular mechanisms that guide and regulate the *de novo* biogenesis of this cytokinetic organelle. Using cytological, biochemical and molecular approaches we have begun to analyze the transport of soluble and membrane proteins to the cell-plate and to characterize the role of the membrane fusion factor, Cdc48p, which has recently been shown to be associated with the cell-plate in dividing cells, in its assembly. These studies will lay the groundwork for further investigations into the complex processes that guide membrane trafficking and fusion during the initial stages of cell-plate vesicle consolidation and the eventual fusion of the expanding cell-plate with the parental plant cell plasma membrane.

PHOTOSYNTHESIS AND RESPIRATION

Panel Manager - Dr. David Oliver, Iowa State University
Program Director - Dr. H. Jane Smith

Towards expanding our knowledge of fundamental aspects of energy capture and utilization by plants, research funded by this program extends from studies on solar energy absorption to analyses of crop productivity. Research supported includes studies on primary events of light absorption, energy transduction and utilization; structure, synthesis, turnover and interactions of components of the photosynthetic and respiratory systems; mechanisms of carbon dioxide absorption and fixation; metabolism and biochemistry of energy-rich compounds; and translocation and partitioning of photosynthates in the whole plant.

9801573 Analysis of the primary electron donor of photosystem I in *Chlamydomonas* mutants

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Grant 98-35306-6601; $105, 000; 2 Years

Crop plants obtain their energy for growth and productivity by converting the energy from sunlight into chemically useful forms in a process termed photosynthesis. Light energy is initially captured and converted into chemical energy in specialized protein complexes termed reaction centers. In plants, two reaction centers, photosystem II and photosystem I, act in series to mediate the oxidation of water and the reduction of compounds subsequently used in later photosynthetic reactions. The objective of this project is to use genetic engineering to alter the photosystem I reaction center complex in a model plant cell system to gain a better understanding of its function, and develop procedures for improving the initial steps of photosynthesis. In photosystem I there exist two potential pathways for electron transfer, one or both of which may be active. We will address how individual amino acids modify the electronic properties of the "special pair" of chlorophylls that function as the primary electron donor such that efficient photochemical electron transfer occurs. Genes encoding the PsaA and PsaB reaction center proteins of photosystem I will be genetically altered to produce proteins with specific amino acid changes. The modified genes will be deposited onto very small gold particles and propelled into the cell by a gas discharge. Inside the cell, the altered genes integrate into the cellular genetic makeup and produce altered reaction center components. Using this approach we will produce a range of different mutant forms of the photosystem I reaction center and investigate how they alter the electronic distribution of the primary donor in the radical state. Analysis of these mutants will allow us to determine if the unpaired electron is located toward the PsaA or PsaB subunit and help identify the active electron transfer branch. Manipulation of reaction center genes will eventually lead to genetically engineered crops with improved photosynthetic capacity and increased productivity.

9801632 Energy Trapping in Purple Nonsulfur Bacterial Membranes

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Grant 98-35306-6396; $170,000; 3 Years

The first step in converting solar energy into chemical energy by plants (photosynthesis) is the absorption of light and the transfer of its energy to a central complex where energy transduction takes place (the reaction center). There are a number of serious constraints that must be overcome by the antenna complexes that serve this function. First, the energy transfer and transduction must occur during the billionth of a second lifetime of the initial excited state. Second, a large fraction of the energy in each photon absorbed must be preserved to drive the photochemistry in the reaction center. Finally, unwanted or destructive photoreactions from the antenna excited states must be avoided in order to both increase the yield of photosynthesis and decrease the yield of undesirable reactions. Our work concentrates on these initial events, using fast time scale spectroscopic measurements, such as picosecond (trillionth of a second) absorbance and fluorescence techniques, to probe the nature of the early states formed by light absorption. We address questions such as, how can the energy transfer and trapping process work so rapidly? How does this energy find its way to and localize in the region of the reaction center? What is it about these excited states and their environment that makes unwanted side reactions unlikely? We are approaching these problems by using a photosynthetic bacterial system, which is a good model for energy transfer and trapping in plants. This facilitates both recombinant genetic and biochemical manipulation, and allows us to utilize the detailed structural information available for the bacterial system.

9801630 The L C-terminus modifies Rubiscos catalytic cycle

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New Investigator Award; Grant 98-35306-6448; $95,000; 2 Years

Ribulosebisphosphate carboxylase/oxygenase (Rubisco) catalyzes a carboxylation reaction and is the enzyme responsible for capture of carbon dioxide from atmosphere. It is a slow enzyme and its carboxylation efficiency is significantly reduced because of competition by oxygen. Either by increasing Rubisco's catalytic rate or by improving its ability to discriminate better between carbon dioxide and oxygen there is great potential for increasing plant productivity. However, it has not yet been possible to engineer Rubisco directly in higher plants. The main objective of this research is to use a chloroplast gene transformation technique to establish a Rubisco active-site-subunit gene replacement system which will allow us to engineer this subunit in higher plants. The second objective is to understand the influence of the evolutionarily diversified C-terminal portion of the active-site-subunit on catalytic properties of the enzyme. The primary engineering of the C-terminus and the mechanistic study of the role of the C-terminus in activity will be performed in photosynthetic bacteria. The data obtained will be finally applied to engineer the higher plant enzyme.

9801685 Molecular Genetics of Nonphotochemical Quenching in Photosynthesis

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New Investigator Award; Grant 98-35306-6600; $120,000; 2 Years

Plants often absorb more sunlight than they are actually able to use for photosynthesis. This extra sunlight poses a problem, because it can cause damage to the plant and thereby decrease crop productivity. However, plants have evolved various ways of protecting themselves against the harmful effects of excess sunlight. This research addresses a major photoprotective mechanism that is found in all plants: the safe dissipation of excess absorbed sunlight as heat (also known as nonphotochemical quenching). The investigation is focused on the characterization of mutants of the model plant, *Arabidopsis thaliana*, that lack the ability to dissipate excess light energy. By determining how the process has gone awry in mutant plants, we will gain insight into how the process occurs in normal plants. Characterization of mutants will involve analysis of the structure and function of light-harvesting protein complexes, which not only absorb sunlight but are thought to be the sites of energy dissipation in leaves. We will identify the gene that is defective in one of the mutants and study the function of the corresponding gene from normal plants. By comparing the physiology and growth of normal and mutant plants, we will evaluate how important it is for a plant to be able to dissipate excess sunlight. Because all plants exhibit this energy dissipation, our findings in *Arabidopsis* will be directly relevant to agricultural crop plants and will lead eventually to increases in crop productivity, especially under adverse environmental growth conditions.

9801618 Identification of Ccb factors required for maturation of holocytochrome b_6

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Grant 98-35306-6975; $150,000; 3 Years

The chemistry of photosynthesis occurs (with very few exceptions) on cofactors, like chlorophyll and heme, which are held in a particular arrangement within a protein. Optimal function of the photosynthetic apparatus (the primary machinery used by nature for fiber, fuel and food production) demands a defined structural arrangement with respect to the cofactor-protein network which in turn requires a specific mechanism for the incorporation of a cofactor into its binding site on the protein. The subject of this project is the question of how a cofactor and a polypeptide are put together in the thylakoid membrane to yield a specific functional arrangement in vivo. It is important to understand these fundamental biochemical mechanisms in order to apply molecular approaches and biotechnology to the solution of agricultural problems relating to yield and energy utilization. Insight into the pathways of cofactor metabolism and utilization contributes also to processes designed to add value to food, fiber and feedstock quality. Cytochrome b_6 is one of the many essential photosynthetic proteins in the chloroplast. It is a membrane protein which contains two functionally distinct hemes within the plane of the membrane. The mechanism by which heme binds to the protein is not known. In previous work, we identified mutants in which the heme protein association was compromised. In this project, we propose to use these or other similar mutants to identify and localize the assembly factors responsible for heme-protein association. In so doing we hope to increase our understanding of a fundamental biochemical problem.

9801782 Characterization of NADP-malic enzyme in a unique Kranz-less C_4 system

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Grant 98-35306-6449; $120,000; 2 Years.

Most crops are C_3 photosynthetic species and thus photorespire. For agriculture this is an inefficient process that increases the need for water, CO_2, and nitrogen. The elimination of photorespiration could substantially improve production efficiency in sustainable systems, lower fertilizer inputs, and reduce environmental impacts on agriculture. Our understanding of the biochemical components and regulation that is required to produce low-photorespiration plants is incomplete. We discovered an aquatic flowering plant. *Hydrilla* (a major weed in southern US), that under adverse conditions changes from a C_3 to a C_4, biochemical system. This eliminates photorespiration, but what is unique about *Hydrilla* is that it does so without using specialized (Kranz) anatomy.. This is crucial, as it circumvents the need to transfer a complex leaf anatomy, which is an advantage in producing more efficient crop plants with C_4-like traits. NADP-malic enzyme is the enzyme which delivers a high CO_2 concentration in the chloroplasts of *Hydrilla*, and thus improves its photosynthetic efficiency. We plan a molecular and biochemical characterization of this enzyme, to understand how it is regulated. The goal is to establish whether it is feasible to transfer C_4 biochemical components to C_3 crops to enhance their performance. *Hydrilla* provides an excellent system to study the induction and regulation of NADP-malic enzyme and how it is integrated into photosynthetic metabolism. From this study we should also learn more about plant responses to CO_2, , and how agricultural plants may be customized to contend successfully with future changes in the global climate.

9801791 Structure and Functions of Photosystem I Light-Harvesting Complexes

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Grant 98-35306-6406;$105,000; 2 Years

Photosynthetic antenna complexes, the most abundant chlorophyll-binding proteins in leaves, precisely assemble pigments and interact with other thylakoid proteins to enhance photosynthetic efficiency by increasing and regulating light absorption and utilization. As such, they are major determinants for crop productivity. Two major classes of light-harvesting complexes, LHC I and LHC II, are specialized respectively for interactions with the photosystem I and photosystem II reaction centers. Because LHC II is more abundant and easily purified, it has been the subject of much structure and function research. Little is known about the structural and regulatory properties of LHC I. One major component, LHCI-730, uniquely is a heterodimer and releases light energy at 735 nm (as opposed to 686 nm for LHC II) at low temperatures. LHCI-730 heterodimers as well as its monomeric subunits can be reconstituted with pigments *in vitro* from apoproteins produced from recombinant genes expressed in bacteria; only heterodimers emit at the long wavelength. Aggregated forms of LHCI-730 emit at the long wavelength at ambient temperatures but most of the absorbed energy is dissipated as heat. In contrast, non-aggregated LHCI-730 exhibit non-quenched, high fluorescence yields at 686 nm. It seems that LHCI-730 can function as either antenna or as a energy sink for protection against photodamage. By manipulation of the sequences of the apoproteins, we study how the chlorophylls responsible for the long-wavelength emission of LHCI-730 are organized and investigate how their environment can be altered by aggregation and other factors to change their spectroscopic and functional properties.

9801577 The Chloroplast H^+ ATPase: Allosteric Subunit Interactions and Tentoxin Binding

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Grant 98-35306-3571; $110,000; 2 Years

Adenosine triphosphate (ATP) is the "energy currency" of all living cells. In plants, the photosynthetic ATP synthase enzyme captures the light energy, which has been converted into the form of a transmembrane electrochemical proton gradient and transforms it into the usable chemical form of ATP. The broad goal of the research to be undertaken in this study is to identify in molecular terms the mechanism of this energy transformation process, and hence to understand factors which govern its energy conversion efficiency. A closely associated goal is to examine and identify the mechanism by which the fungal product tentoxin binds to and inhibits the function of the chloroplast ATP synthase. Tentoxin is a plant-specific inhibitor and thus has significant potential use as a natural and highly specific herbicide. This work involves application of a combination of genetic engineering and biochemical/biophysical approaches to a) probe structural changes which take place in the photosynthetic ATP synthase enzyme during ATP production, and b) probe the specific binding site of the fungal inhibitor tentoxin. Part of the complex structure of the catalytic F_1 (factor 1) segment of the ATP synthase has been solved to high resolution by x-ray crystallography. Using the information provided by this structure we are altering specific amino acid residues in the a, b and g protein subunit genes of the spinach chloroplast F_1 and following the effects of the changes on the function of the mutant proteins. The work also involves genetically engineering sites into the enzyme for specific attachment of chemical probes which will provide information relating to the dynamics of the energy interconversion process.

9801689 Components of the Microalgal CO_2 Concentrating Mechanism

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Grant 98-35306-3570; $120,000; 2 Years

The long term goal of our research is to acquire a better understanding of how single-celled algae (microalgae) concentrate carbon dioxide (CO_2) internally when grown with air levels of CO_2. This CO_2 concentrating mechanism (CCM) involves an energy-requiring "pumping" of CO_2 and is responsible for an increased efficiency of photosynthesis in microalgae.. Most higher plants, in which photosynthesis is CO_2-limited, do not have the capability to concentrate CO_2, and the mechanism used by those that do is more complex than the algal mechanism. The specific goals of this project are identification and characterization of algal mutants with defects in functional components of the CCM, and cloning of the corresponding genes. A complete understanding of the algal CCM, including identification of the genes involved, will allow us to evaluate the feasibility of transferring the CO_2 concentrating capability to agriculturally important plants. Expression of a CCM in such plants should give rise to more efficient photosynthesis, potentially increasing yields and/or water use efficiency. The CCM is an essential part of the photosynthetic system of microalgae and cyanobacteria (blue-green algae), organisms that play a major role in the carbon balance of the biosphere. Thus, understanding the function and regulation of the CCM is important, not only for evaluation of the potential for the transfer of this system into agriculturally important plants, but also to increase our understanding of photosynthesis and its regulation in these ecologically important groups of photosynthetic organisms.

9801686 An analysis of sieve element plasma membrane proteins using monoclonal antibodies

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Grant 98-35306-6591; $105,000; 2 Years

Photosynthetic products made in leaves are transported through the plant body by specialized cells called phloem sieve elements. The movement of sugars and amino acids from leaves to the grains, fruits and tubers that are harvested from crop plants is a process that is critical to agriculture. The phloem sieve element is a unique kind of cell. During development sieve elements lose their nuclei, vacuoles and most other cytoplasmic components. Large pores develop in the cell walls that join the cells together, and the sieve elements form a lengthy, "hollow," tube through which nutrient solutions flow. Unlike corresponding water-conducting cells of the xylem tissue, however, phloem sieve elements remain alive at maturity. The sieve element plasma membrane remains intact in mature cells. Because the mature sieve element lacks a nucleus and ribosomes, it also lacks the ability to manufacture proteins. Identifying the proteins present in the plasma membranes of mature sieve elements is a critical step in learning how these cells function. We will produce phloem-specific monoclonal antibodies by immunizing mice with phloem cells isolated from plant tissue cultures and screening the resulting mouse hybridoma colonies using an immunofluorescence microscopy procedure that identifies labeled phloem cells using free-hand sections of plant stems. The phloem-specific monoclonal antibodies will be used to identify sieve element-specific proteins using western blots and other biochemical procedures. The sequence of amino acids in the proteins identified by the phloem-specific monoclonal antibodies will be determined to identify the nature and possible functions of the phloem proteins.

9801783 The Roles of Photosystem I Cyclic Electron Transport in Stress Tolerance by Photosynthetic Organisms.

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Strengthening Award; Grant 98-35306-6602; $90,000; 2 Years

Photosynthesis provides all the energy required for plant growth. The first part of photosynthesis consists of an electric current that is driven by sunlight. This current generates the reducing and phosphorylating compounds that are used by plants to manufacture carbohydrates, oils, proteins, and other materials. Most of this current of electrons follows a well-defined linear path through the photosynthetic membranes of a leaf. A small fraction of the electrons are diverted into a cyclic path, however. This cyclic path is universal in plants; how it contributes to plant growth is unknown. Recently, several research groups have proposed that the cyclic path of electron flow helps plants survive environmental stresses such as cold, drought, lack of mineral nutrients, or excessive sunlight. Our project will test this hypothesis using genetic and physiological approaches in the photosynthetic bacterium *Synechocystis*. *Synechocystis* performs photosynthesis in the same way crop plants do but is easier to work with in the laboratory. Our approach will be to turn off genes that make components of the cyclic path of electron flow and then observe if sensitivity to cold, strong light, or lack of mineral nutrients is increased. The genome of *Synechocystis* has been fully sequenced and is freely available on the Internet, facilitating this approach. The long term goal of this research is to understand the mechanisms by which plants tolerate environmental stress. Understanding these mechanisms will allow genetic engineering of stress-tolerant crop plants that give better yields under adverse or unpredictable weather and soil conditions.

9801634 Structural Studies of Cytochrome b_6f and Component Proteins by X-ray Crystallography

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Grant 98-35306-6405; $140,000 ; 2 Years

Photosynthesis is the remarkable conversion of light energy to chemical energy by which living cells carry out life processes. The light-to-chemical energy conversion is done by splitting water into protons, electrons and oxygen molecules. Chemical energy is accumulated as the protons and electrons pass through several proteins of the photosynthetic machinery. This study aims to understand how some of these protein molecules capture and transfer protons and electrons. Protons and electrons pass through several complex protein "machines" during energy conversion. This project is focused on cytochrome b_6f, the protein machine that carries electrons between the two light-absorbing protein machines of photosynthesis. Proteins are very large, complicated molecules with thousands of atoms. Despite their large size, most proteins have a well defined structure, which is critical to their biological function. Great advances in understanding biological processes come from knowing the structures of the proteins that carry out the processes. Therefore, we are determining structures for the proteins of cytochrome b_6f. The first objective of the current project is to determine the structure of intact cytochrome b_6f, which includes four different proteins. With earlier USDA support, we determined the structures of the cytochrome f and Rieske proteins of cytochrome b_6f, and these will be used to build a picture of the functioning, intact complex. The path for protons through cytochrome b_6f is not well understood, and our second objective is to investigate a possible pathway for protons through cytochrome f.

9801636 8th International Conference on *Chlamydomonas*

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Genetics Society of America; Bethesda, MD 20814
Grant 96-35306-3914; $4000; 1 Year

This proposal requests support for the 8th International Conference on the Cell and Molecular Biology of *Chlamydomonas*, to be held in Tahoe City, California on June 2-7, 1998. Since its inception in 1983 this now biennial conference has brought together investigators from different disciplines from the United States and abroad to discuss a variety of problems being studied in this organism including organelle heredity, cell differentiation and motility, fertilization, nitrogen and carbon metabolism, resistance to drugs and herbicides, and photosynthesis. The chloroplast of *Chlamydomonas* is very similar to that of higher plants and photosynthetic measurements are easily made on intact cells or thylakoid membrane preparations. In addition, mutants blocked in photosynthesis are readily obtained and are viable if cells are provided acetate as a carbon source. Experiments with *Chlamydomonas* often have paved the way for progress in many areas of chloroplast and photosynthesis research in agriculturally important plants. The interactions between scientists presenting their work at this conference have been particularly useful in rapidly disseminating technical breakthroughs and developing new, broadly useful methodologies. The meeting also has been important in establishing collaborations and in promoting exchange of mutants and molecular probes. Finally, the continued recruitment of new investigators with different backgrounds and fresh ideas is critical for maintaining the vitality of the field. This conference makes it possible for graduate students and postdoctoral fellows to be exposed to the full range of studies of *Chlamydomonas* and also to have the opportunity to meet colleagues and potential postdoctoral mentors.

9801579 Charge Separation and Stabilization in Chloroplast Photosystem II

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Grant 98-35306-6751; $195,000; 3 Years

Plants use water as the ultimate electron donor for the photochemistry that drives photosynthesis. The electrons and protons that are removed from water by the reaction center chlorophyll in this process are eventually used to convert carbon dioxide to starch, proteins, nucleic acids, and other valuable biomolecules. The fact that plants have evolved so as to use water in this capacity accounts for the global proliferation of photosynthesis. At present, the mechanism by which plants catalyze this remarkable chemistry is uncertain; but, with past USDA NRICGP support, we have made significant progress in developing a mechanistic model for oxygen generation. This model proposes that the electron that is removed from chlorophyll by light is replaced by one coming from an amino acid called tyrosine. The tyrosine, in turn, removes a proton and an electron from a water molecule that is bound to a cluster of four manganese atoms in the protein matrix. This hydrogen-atom abstraction process is repeated four times to strip four protons and four electrons from two water molecules and release a molecule of oxygen. This model accounts for a great deal of the observations that have been made on photosynthetic oxygen generation and has generated considerable interest in the photosynthetic field. The ongoing research that we plan, with USDA support, is aimed at testing this model further by studying the chemical structure of intermediates that occur in this process and by investigating the role of the calcium and chloride cofactors that are also required for photosynthesis.

9801617 Regulation of Solute Exchange Between Sieve Elements and Companion Cells

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Grant 98-353-6-6434 $105,000, 2 Years

Plant productivity in agriculture depends on transport of food from leaves to other organs such as seeds and roots. Food transport takes place in the phloem, a complex and little-understood tissue. The food conducting cell, the sieve element, is supported in its metabolism and function by its companion cell. Companion cells are largely responsible for accumulating the sugars that are made in leaves and transported by the sieve tubes. Although complex interactions between companion cells and sieve elements has always been assumed, techniques are only now becoming available to study these interactions in detail. We propose that exchange of sugars and other small molecules between companion cells and sieve elements is more selective than previously realized. If this is so, attempts to introduce compounds such as new sugars or insect feeding deterrents into the phloem will require engineering of transport functions as well as synthesis. To test our proposal we will use a gene promoter (the galactinol synthase promoter from melon), cloned during the previous grant period. This promoter is expressed specifically in the companion cells of minor veins in *Arabidopsis* leaves. Will use this promoter to direct expression of novel compounds such as galactinol and octopine in minor vein companion cells of transgenic *Arabidopsis* plants. We will then use conventional transport physiology techniques to monitor entry and translocation in the sieve tubes.

9801640 Dynamic Physical and Biophysical Interactions Surrounding the Photosystem I Iron-Sulfur Clusters

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Grant 98-35306-6476; $160,000; 2 Years

Green plants contain two chlorophyll-containing photosystems which operate in series to convert sunlight into chemical energy. These two photosystems contain many similar components in the earliest stages of photochemical charge separation, but they differ at one crucial step. In photosystem II, this step occurs near a single iron atom and it allows the electron to remain on a quinone, but in photosystem I, this step involves the participation of a cubic iron-sulfur cluster. This difference allows the photochemically-promoted electron in photosystem I to be vectored out of the reaction center for the purpose of reducing NADP+. In this research, we combine molecular genetics with biophysics and biochemistry to study two aspects of electron transfer through $F_{x.}$: the magnetic orientation of the F_x cubane relative to the polypeptide backbone, and the equilibrium constant between the preceding acceptor, A_1 and F_x These structural and functional issues will determine whether the location of the mixed- and equal-valence pairs has relevance to the rate of electron transfer, and whether the midpoint potential of the mixed-ligand $C5655_{psa}$ and $C556_{psb}$ clusters is the reason for the low efficiency of forward electron transfer from A_1. Because a detailed understanding of the mechanism of photochemical energy conversion is essential for attempting to increase the efficiency of photosynthesis in plants, this project has relevance to long range-improvement and sustainability of agriculture. The knowledge gained by this research should help guide efforts to manipulate and optimize photosynthetic efficiency in crop and forest plants.

9801446 1998 Gordon Research Conference on Mitochondria and Chloroplasts

Stern, D.
Gordon Research Conference; West Kingston, RI 02892-0984
Grant 98-35306-6477; $7,000; 1 Year

The Gordon Conferences cover many aspects of science, and are known by their motto, "the frontiers of science." These meetings accept relatively few participants and feature open discussions and a convivial atmosphere, where internationally-known scientists mix freely with young investigators, students and postdoctoral fellows. The conference funded by this proposal covers mitochondria and chloroplasts, two essential energy-producing compartments in eukaryotic cells. When mutations occur that affect the expression of mitochondrial or chloroplast genes, the result is often reproductive or fatal disorders, both in animals and plants. This conference will highlight areas where recent progress has been most impressive, and areas that were under represented in the last conference in this series. These subjects include organelle transmission, mitochondrial mutations, and interorganellar interactions. Information exchanged here will be largely fundamental in nature, however it will also be directly applicable to genetic engineering, particularly with respect to chloroplast modifications, a highly active area in the biotechnology sector. USDA funds will specifically be used to fund travel of U.S. scientists to this meeting, supporting a mix of young scientists and more established ones. Without these funds, U.S. representation at this European meeting would undoubtedly be greatly diminished.

9801619 Metabolic Engineering of Plants for Enhanced Productivity

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Grant 98-35306-6469; $110,000; 2 Years

Plant productivity is governed by the capacity of leaves to fix carbon dioxide to make sugars by photosynthesis and the utilization of these sugars for growth and development by sink tissues. Much of the effort to increase plant productivity has been directed at the photochemical processes involved in the conversion of light into chemical energy, and at the enzymological process of carbon dioxide fixation or the competitive reaction with oxygen catalyzed by ribulose bisphosphate carboxylase. Although improvement in these primary assimilatory processes are fundamental in increasing plant productivity, the utilization of these sugars and their conversion into starch and oils in developing sink organs such as seeds and tubers are also important. The overall objective of this grant is to evaluate the impact on photosynthesis when the capacity of leaves or developing seeds to convert sugars into starch is increased. Genetically engineered rice plants expressing a modified enzyme for starch synthesis in seeds will be evaluated for their seed weight production and their potential for net photosynthesis. Genetically engineered *Arabidopsis* plants expressing a modified enzyme for starch synthesis in leaves will be evaluated for their overall capacity for net photosynthesis and partitioning between sugars and starch under a variety of environmental conditions. These studies will aid in efforts to increase productivity of crop plants to levels not attainable by traditional breeding programs.

9801641 Regulated Enzymes in CO_2 Fixation: Mechanisms of Activation & Catalysis

Miziorko, H.M.

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Grant 98-35306-3448; $140,000; 2 Years

Long term aims of this project include investigation of the photosynthetic apparatus involved in converting CO_2 into carbohydrate. Previously, progress in this area has been made by studying isolated enzymes that are components of the photosynthetic apparatus. This approach will be continued; the enzyme phosphoribulokinase is the current focus of this project. Phosphoribulokinase is a regulated enzyme; its activity influences the rate of carbohydrate formation in plants and related carbon fixing organisms. A recombinant form of this enzyme is being engineered to identify amino acids important to activity and, by replacing them, to establish how they are important to enzyme function. The recombinant form of the enzyme has also been used in collaborative work that has led to elucidation of the enzyme's structure (at atomic resolution). Proposed work includes protein engineering and mutagenesis studies aimed at identification of new components of the catalytic apparatus as well additional functional assignments of crucial amino acids that are situated within the enzyme's active site. Pursuit of collaborative work aimed at generating improved high resolution structural data on this key photosynthetic enzyme is also planned.

NITROGEN FIXATION/NITROGEN METABOLISM

Panel Manager - Dr. Teresa Thiel, University of Missouri-St. Louis
Program Director - Dr. H. Jane Smith

Grants in this program support research which will improve our understanding of how nitrogen is fixed biologically, metabolized and cycled by crop plants. These goals are of importance to sustainable plant production, since availability of nitrogen is a common limiting factor in plant growth, and because of costs associated with application of nitrogen fertilizers. Areas supported by the program include, but are not limited to: chemistry of biological nitrogen fixation; factors limiting biological nitrogen fixation; plant-microbe interactions involved in establishing and maintaining the biological-nitrogen fixation symbioses; and metabolism of nitrogenous compounds by higher plants.

9803500 Genetic Analysis of Enhanced Nitrogen Fixation by a Symbiotic Cyanobacterium
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Grant 98-35305-6748; $90,000; 2 Years

This project focuses on genetic analysis of heterocyst differentiation and nitrogen fixation in the cyanobacterium (blue-green alga) *Nostoc punctiforme*. Heterocysts are cells specialized for protecting the nitrogenase enzyme against inactivation by air. Heterocysts typically form at a low frequency (less than 10% of the cells) when the environment is limiting in combined nitrogen. However, in symbiosis with certain plants, *N. punctiforme* differentiates heterocysts at a higher frequency (up to 45% of the cells), fixes nitrogen at a higher rate and releases ammonium for growth of the plant. Our ultimate goal is to identify and manipulate the genetic mechanisms regulating these processes, such that the symbiotic phenotype can be duplicated in the absence of the plant, thereby allowing for conditional release of fixed nitrogen by Nostoc species in aquatic agricultural systems. We have developed techniques and experimental tools for genetic analysis of *N. punctiforme* and will screen transposon-induced mutants for a heterocyst-minus phenotype. To verify the operation of a symbiotic regulatory system, heterocyst-minus mutants will then be screened for symbiotic nitrogen-fixation with the plant partner *Anthoceros punctatus*. Secondary mutants of heterocyst-minus, symbiotic-positive mutants will be positively selected by growth on nitrogen gas in air. A newly identified gene that is essential for heterocyst differentiation, *hetF* will be characterized by domain deletion analysis, and epistatic relation with the early regulatory gene *hetR*. Biosynthesis patterns of regulatory genes in the symbiotic growth state will be determined by a reporter gene (*lacZ*) fused to the gene of interest, coupled with antibody assays.

9803558 Mechanism of Nitrification in *Nitrosomonas europaea*: Structure of Cytochrome P460
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Postdoctoral Fellowship; Grant 98-35305-6549; $90,000; 2 Years

The availability of fixed nitrogen or ammonia in the soil is one of the critical factors that limits plant productivity. Ammonia is the preferred form of nitrogen in the soil because of its stability. As a consequence, more than 6 million tons each of urea and ammonium nitrate are produced in this country every year with a cost of about $100 to $150 per ton. Greater than eighty percent of this product is used for fertilizers. The process of "nitrification" occurs readily in well drained soils common to much of America's farmland. This process is catalyzed by a select group of bacteria that depletes the fixed nitrogen by converting ammonia to nitrite and then nitrite to nitrate. Nitrate is rapidly leached from the soil during heavy rainfall or watering. Not only is this unfavorable with respect to the loss of expensive fertilizer, but leached nitrate can also pollute local waterways. To prevent this, chemicals such as nitrapyrin (2-chloro-6-trichloromethyl pyridine) have been employed to inhibit or stop this process. However, the release of chloro-aromatic compounds into the environment may have consequences that have not yet been realized. In order to better understand the mechanism of nitrification we are using X-ray diffraction techniques to solve the three dimension structures of some of the enzymes involved in this process. This information will be fundamental to the design of effective and environmentally safe inhibitors of nitrification.

9803543 Are Lectins Involved in Legume Nodulation?
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Grant 98-35305-3583; $85,000; 2 Years

Legumes are the only plant family (with one notable exception, *Parasponia* in the elm family) that establish symbiotic associations with nitrogen-fixing *Rhizobium* bacteria. Indeed, within the legume family itself, there is a great deal of host specificity; alfalfa is nodulated by *Rhizobium meliloti* and soybean by *Bradyrhizobium japonicum*, and not vice versa. Based on the strong correlation between host specificity and the ability of plant carbohydrate-binding proteins known as lectins to bind to *Rhizobium* cells, the lectin-recognition hypothesis was formulated more than 20 years ago. We have verified the hypothesis by introducing the soybean lectin

(*SBL*) gene into Lotus corniculatus which is normally nodulated by *R. loti;* such plants are now nodulated by *B. japonicum*. Although our results indicate that lectins are indeed critical for host recognition, the nodules produced on the transgenic *Lotus* plants were deficient in nitrogen fixation because the rhizobia did not colonize the root nodules. One possibility is that more than one lectin is required for proper entry into the "wrong" host. We propose to 1) isolate and characterize *Mslec3*, the third lectin gene from alfalfa; *Mslec1* and *Mslec2* from alfalfa were identified earlier; 2) identify other lectin-like proteins in alfalfa, particularly those with a kinase domain that could function as a receptor for a rhizobial ligand; and 3) introduce these lectin genes singly and in combination into the genetically tractable but non-nitrogen-fixing plant *Arabidopsis* as well as into *Lotus* to determine whether these genetically modified plants become colonized by *Rhizobium meliloti*.

9803557 Soybean Nucleobase Transporters

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Strengthening Award; Grant 98-35305-6749; $110,000; 2 Years

Soybean is one of the few crop plants that hosts a symbiotic relationship with nitrogen-fixing bacteria. In plant root nodule cells resident bacteria capture atmospheric nitrogen and change it into a form useful to plants. Available nitrogen is then converted into ureides, the major nitrogen transport molecules in soy bean. This complex process involves the exchange of ureide precursors (or nucleobases) between nodule cells and within compartments of nodule cells. Little is known about the membrane transport proteins that control this essential cellular metabolite traffic. We are investigating nucleobase transporters and their role in ureide synthesis. Information gained from such studies will clarify current ambiguities in ureide synthesis and is directly relevant to future genetic engineering of soy bean for efficient nitrogen assimilation. We propose to isolate soybean nucleobase transporter genes through two methods. Soybean nucleobase transporter genes will be cloned by conventional molecular techniques using recently complied DNA information from other plant nucleobase transporter genes. In addition, soybean transporter genes will be cloned through genetic complementation of a yeast strain deficient in ureide transport. Genes will be sequenced and analyzed for tissue, developmental and cell-type expression patterns. The membrane localization of the encoded nucleobase transporter proteins will be investigated using epitope tag technology, nucleobase transporter-specific antibodies and immuno-histological techniques.

9803544 Functional Properties of Spinach Nitrate Reductase

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Grant 98-35305-3542; $105,000; 2 Years

The enzyme assimilatory NADH:nitrate reductase catalyzes the rate-limiting and regulated step in the pathway of inorganic nitrogen assimilation which is utilized by plants to convert nitrogen into a biologically useful form (ammonia) for growth. The proposed research will combine an extensive array of biophysical techniques, such as initial-rate kinetics, W/visible absorption, CD, fluorescence and NMR spectroscopies and real-time biomolecular interaction analysis with molecular biological methods that include recombinant protein expression and oligonucleotide site-directed mutagenesis to probe the interaction(s) between the flavin- and heme-containing domains in spinach nitrate reductase. Molecular modeling, will be combined with chemical crosslinking and peptide sequencing experiments to identify specific amino acid residues in the functional flavin and heme domains that are responsible for stabilizing domain-domain interactions which will be subsequently confirmed by directed mutagenesis. Studies will focus on specific amino acid residues in both the purified flavin and heme domains and the flavin/heme fusion protein. Binding constants will be determined to assess the relative strengths of the domain-domain interactions and monitor the kinetics of the association and dissociation processes. These studies will assist in identifying structural features of the enzyme that are critical for both catalysis and stability and essential for plant growth and have the potential for enhancing agricultural productivity.

9803542 Role of Microbial -Glucans in Soybean Nodule Development

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College Park, MD, 20742-4450
Grant 98-35305-6590; $110,000; 2 Years

Plants respond to structurally diverse carbohydrate molecules from microorganisms during symbiotic and pathogenic encounters but their roles remain to be elucidated. Bacterial mutants defective in the synthesis of periplasmic polysaccharides, -glucan, are impaired in symbiosis or virulence. We have isolated and characterized the -glucan synthesis locus from a microsymbiont of soybean, *Bradyrhizobium japonicum*. Two genes from *B. japonicum* have been identified (*ndvB,C*), which are required for glucan synthesis as well as for symbiotic N-fixation in association with soybean. We propose to create specific mutations by amino acid substitution at the catalytic site of the enzymes synthesizing glucans. Synthesis of structurally altered -glucans by the mutant strains will provide insights into their structure-function specificity for symbiotic plant-microbe interactions. Objectives: (I) Determine the effect of introduced mutations in the active site of NdvB (which we predict to be a processive glycosylase) on -glucan structure and function. (II) Identify and clone genes induced (or repressed) in *B. japonicum* due to defects in -glucans. These studies will help to elucidate the role of cyclic -glucans during symbiosis and should provide fundamental advances in the understanding of symbiotic and pathogenic plant-microbe interactions.

9803538 Role of Protein Phosphatase 2C in *Lotus japonicus* Root Nodule Development
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Grant 98-35305-6551; $120,000; 2 Years

Nitrogen is the one of the most important elements of our biosphere. Dinitrogen (N_2) constitutes 80% of the air, but cannot be utilized by higher organisms, including plants, until it is converted into ammonia or nitrate. A variety of microorganisms, however, are capable of dinitrogen reduction or fixation. A particular subgroup of nitrogen-fixing bacteria, the rhizobia, establish a highly evolved beneficial interaction with specific plants and fix atmospheric nitrogen into ammonia inside plant cells, in a novel, specialized plant organ called a "nodule". The ammonia thus produced is assimilated by the plant and used as nitrogen source for growth. The process of rhizobial infection of plant roots, nodule ontogeny and symbiotic nitrogen fixation are highly controlled processes. Multiple genes of both rhizobial and plant origin are activated at specific stage of the nodulation process. We are interested in elucidating the signal transduction pathway responsible for activating a particular class of symbiotically regulated plant genes, expressed late during nodule development (late nodulin genes). In this project, we will analyse the function of a plant gene encoding a protein phosphatase 2C enzyme, isolated from the model legume *Lotus japonicus*, which we postulate to be an important component of the regulatory circuit controlling late nodule development and functioning. This type of research is not only important for developing a basic understanding of the nitrogen fixing symbiosis and plant-microbe interactions, but has also a number of applied aspects, including the reduction of environmental contamination with excess nitrates derived from chemical fertilizer, and the long term objective of extending the symbiosis to presently non-nodulating plants.

9803562 Structural and mechanistic analyses of urease and allantoinase
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Grant 98-35305-6550; $140,000; 2 years

Crops and other plants transform nitrogen-containing compounds found in soil (such as fertilizer-derived urea) or produced within the plant (such as allantoin) into building blocks that are used for plant growth. We plan to study the proteins urease and allantoinase, two of the key enzymes in this network of reactions. Continued investigation of the structure and mechanism of action of urease will provide groundwork for the design of agriculturally significant urease inhibitors. These inhibitory compounds could be used to reduce the toxicity and increase the efficiency of urea-based fertilizers. Similar efforts will be carried out with allantoinase in order to understand how the plant metabolizes allantoin, a critically important compound used for distributing nitrogen from the roots to other tissues in many plants.

9803598 Alanine, not ammonia, is excreted from *Bradyrhizobium japonicum* bacteroids
Emerich, D.
University of Missouri; Department of Biochemistry; Columbia, MO 65211
Grant 98-35305-6909; $50,000; 1 Year

Nitrogen is the most limiting nutrient in agriculture with the occasional exception of water. The major input of nitrogen into the biosphere is through a process called biological nitrogen fixation. Biological nitrogen fixation is catalyzed by a relatively few species of bacteria. The contribution of free-living bacteria to the total nitrogen economy is small. The major input of biological nitrogen is via bacteria in association with plants. An example of this association is the soybean plant and its bacterial partner, *Bradyrhizobium japonicum*. The soybean and the bacteria form a unique symbiosis in which the plant provides the bacteria with carbon compounds produced from photosynthesis and in return the bacteria reduces atmospheric nitrogen gas into a form that the plant can use. Ammonia is the form of nitrogen believed to be given to the plant by the bacteria via the simple process of chemical diffusion. The diffusion process can not be directed to a specific location and requires a large concentration within the bacteria to be efficient. We have found that an amino acid, alanine, is probably the actual form of nitrogen that is transported to the plant. Using alanine rather than ammonia means that the process is under biological control rather than at the mercy of chemical diffusion and thus can be directly transported out of the bacteria and does not require a large concentration to be efficient. This proposal will attempt to better characterize the alanine biosynthesis and transport process.

9803599 Specificity in the soybean-*Sinorhizobium* symbiosis: Type III protein secretion
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Grant 98-35305-6750; $120,000, 2 Years

Biological nitrogen fixation by *Rhizobium* provides millions of pounds of free fertilizers each year to crops such as soybean. Root nodules, which house the rhizobia, capture nitrogen from the atmosphere and channel it into plant metabolic pathways in an environmentally sustainable manner. However, biological nitrogen fixation is relatively inefficient, and so far, it has benefitted little from the techniques of molecular biology. Work in our laboratory centers on soybean-Sinorhizobium symbiosis. We have identified a protein secretion system that releases proteins from the cells into the rhizosphere. We have evidence that these released proteins may have an important role in determining the fate of symbiosis. We are planning to characterize the extra cellular proteins and their secretion system, so that we can better understand their roles in symbiosis. Information obtained by these studies will enable us to rationally manage and enhance the process of biological nitrogen fixation with soybean.

9803554 Soybean Urate Oxidase and the Ureide Pathway

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Grant 98-35305-6548; $160,000; 2 Years; 1998 Award: $105,000

Nitrogen is a limiting nutrient for most crops; legumes such as soybeans occupy a special place in agriculture because they possess the ability to convert atmospheric nitrogen into ammonia. The metabolic pathway by which the ammonia is converted into organic compounds which can be used by the plant to support its growth is called the ureide pathway. Nitrogen fixation is an energetically costly process for the plant, and a worthwhile goal for agricultural research is to enhance the efficiency of the process by which nitrogen becomes available to the plant in usable form. A prerequisite for doing so, however, is to gain a detailed understanding of the ureide pathway and its component enzymes. One of the enzymes in the ureide pathway isurate oxidase, which catalyzes the conversion of urate to 5-hydroxyisourate. It is not known how 5-hydroxyisourate is converted to allantoin, which is the metabolite that the plants transport from the roots, where nitrogen fixation and the urate oxidase reaction occur, to the stem and leaves, where the nitrogen is used in amino acid biosynthesis. One goal of our research program is to characterize a new enzyme that may be involved in the conversion of 5-hydroxyisourate to allantoin. We have purified the novel enzyme and one of our current goals is to clone its gene. Our second goal is to examine the chemical reaction catalyzed by urate oxidase using techniques that allow us to trap the intermediates of the reaction as they are formed on the enzyme.

9803545 Gordon Research Conference on Nitrogen Fixation

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Grant 98-35305-6475; $8,250; 1Year

Although the focus of this Gordon Conference is on the reactions associated with biological nitrogen fixation, with particular emphasis on the function of nitrogenase, the enzyme which catalyzes the biological reduction of N_2 to NH_3, lectures will also cover related topics of hydrogenases, the mechanism and assembly of urease and new and provocative systems for nitrogen fixation. Five specific areas of nitrogen fixation research will be covered: 1) Regulation of expression of nitrogenase, 2)biochemical mechanism of nitrogenase, 3)chemistry of nitrogen reduction, 4)metallocenter assembly and 5)metabolism of nitrogen. In pursuit of Gordon Conference goals of fostering open exchange of ideas, the conference will be organized to provide ample time for discussion after each presentation. Young scientists, graduate students and postdoctoral fellows will chair discussions of poster sessions. Funds of this award will be used to support travel expense for these young scientists.

9803503 AgNOD-GHRPs, unique nodulins expressed in the early stages of nodule-cell infection.

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Grant 98-35305-6554; $85,000; 2 Years

A primary objective of this project is to discover processes by which nitrogen-fixing soil bacteria are able to infect the roots of selected host plants and establish ecologically and economically valuable root nodule symbioses. In these symbioses, molecular nitrogen, abundant in the atmosphere in a relatively inert form, is converted in a process called nitrogen-fixation to a usable form which is transferred directly to host plants in a highly regulated process that does not contribute to environmental degradation. Furthermore, symbiotic biological nitrogen fixation is accomplished with the use of solar energy in a process far more efficient than the production of chemical nitrogen fertilizers. At a time when farmland and wildlife habitats are decreasing at an alarming rate, when soils are becoming less productive and water sheds increasingly contaminated by heavy use of chemical fertilizers, it is critical to American agriculture to increase the use of biological nitrogen-fixing systems. In order to determine the genes required for symbiosis between the nitrogen-fixing actinomycete *Frankia* and its host plants, we are studying proteins expressed specifically in nitrogen-fixing root nodules. Unique metal-binding proteins, GHRPs have been identified within these nodules and their metal-binding capacity and specificity are being studied, as well as their basic physical and biological properties. These studies will contribute to an understanding of the genetic and biochemical components required for symbioses and will provide fundamental new knowledge about biological nitrogen-fixing systems that will lead to their increased use in agricultural and in soil stabilization and reclamation.

9803493 Arrest of rhizobial infections in *Medicago truncatula*

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Grant 98-35305-6686; $90,000; 2 Years

The bacterium *Rhizobium* infects plants in the legume family, causing the formation of root nodules where bacteria convert nitrogen gas to ammonium. This symbiosis satisfies the plant's nutritional requirement for nitrogen and enriches the soil. My laboratory studies the infection of roots by *Rhizobium meliloti* and its regulation by the host plant *Medicago truncatula*. This close relative of alfalfa is particularly suitable for genetic and molecular analysis. Although this bacterial infection is beneficial to the plants, it uses much of the plant's nutrients and energy resources. Therefore, the plant strictly governs the numbers of infections that occur and that persist to form nodules. We are using plant mutants that fail to form successful infections to (1) identify plant genes required for maintenance of the bacterial infection, and to (2) study the means by which the plants restrict uncontrolled growth of the bacteria. The work to be carried out under this award will characterize several different mutations that affect infection of legumes by *Rhizobium*, and will work towards understanding the manner in which the affected genes function together to control infection. We will also investigate whether the plant uses defense responses -natural mechanisms that are used to control and eliminate pathogen infections - to control infection by this beneficial bacterium. We expect that continued work in this area will lead to mapping and cloning of these 'symbiosis' genes. In addition to defining factors required for improvement of the plant's nutritional status, the studies will contribute to the general understanding of plant developmental mechanisms, including cell growth, proliferation, and death.

9803559 Structural Analysis of the Gene Products Involved in FeMo-cofactor Biosythesis.

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New Investigator Award; Grant 98-35305-6552; $100,000; 2 Years

The biological conversion of atmospheric nitrogen to ammonia or nitrogen fixation is of paramount importance for global plant productivity. This process only occurs within microorganisms, either free living or in a symbiotic association with certain leguminous plants. The enzyme components that carry out nitrogen fixation termed nitrogenase have been characterized at the atomic level, however, the reaction mechanism is not yet understood. It is known, however, that the process involves a unique cluster of metals bound to the enzyme. We are studying the biological assembly of this complex cluster of metals to understand better their involvement in the catalytic mechanism as well understand how this cluster is synthesized. This information may be applied to development of more economic and environmentally-friendly chemical alternatives to industrially produced nitrogenous fertilizers. The approach we are using (structure determination by x-ray diffraction methods) allows use to visualize enzymes and their associated metal centers to atomic detail. The information is vital in creating models that can be approached experimentally for metal cluster assembly and nitrogenase-catalyzed nitrogen fixation.

9803601 The Mechanism of Nitrogenase Catalysis

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Grant 98-35305-6687; $100,000; 2 Years

Nitrogen in a form capable of supporting plant and animal growth is essential for agricultural productivity as well as maintaining the earth’s various ecological cycles. This "fixed nitrogen" arises from natural processes such as lightening discharges and natural or man-made fertilizers, but most fixed nitrogen enters the biosphere through the process of biological nitrogen fixation. This process is part of the earth's natural nitrogen cycle and is carried out by the nitrogenase enzyme found in a few types of naturally occurring bacteria found in the soil and oceans. The nitrogenase enzyme consists of two separate proteins which together catalyze the conversion of atmospheric nitrogen into ammonia by the following reaction $N_2 + 6e + 6H^+ = 2NH_3$. The NH_3 is then converted into various biomolecules, such as DNA, proteins, and cofactors that are essential for all life processes. Thus, nitrogenase commands a key position in sustaining the thread of life that depends on fixed nitrogen sources. To understand its biological requirements, its mechanism of action and its vulnerabilities is key to controlling biological nitrogen fixation, *via* nitrogenase, for improved agricultural and ecological benefits. A complete and detailed understanding of nitrogenase catalysis is essential and requires a continued research effort. The proposed research will provide a better understanding of how nitrogenase catalyzes the conversion of atmospheric nitrogen by providing a step-by-step view of the catalytic process.

9803499 *GlnB* in Methanococcus

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Grant 98-35305-3891.$100,000; 2 Years

A wide variety of microorganisms (bacteria and bacteria-like organisms called *Archaea*) are able to carry out the important process of nitrogen fixation. Our laboratory is investigating nitrogen fixation in a group of microorganisms called the methanogenic

Archaea. These organisms have not been studied as thoroughly as many other nitrogen fixing organisms. However, because they evolved along a completely different line than more "familiar" organisms, novel findings are likely to be made. In addition, the methanogenic *Archaea* may be found in a unique set of agriculturally important habitats. They are anaerobic (unable to live in the presence of air), and are found in oxygen-free pockets in soils and in certain bioreactors where organic waste is converted to the fuel methane. Our laboratory has discovered the presence of a potentially important set of genes in a species of methanogenic *Archaea* called *Methanococcus maripaludis*. These genes belong to a family called *glnB* that is also found in more familiar nitrogen metabolizing organisms and are likely to play a role in the regulation of nitrogen fixation. Our goal is to determine what this role is. To accomplish this goal, we have the technology to make mutations in these *glnB*-like genes and to determine how these mutations affect the expression of the nitrogen fixing function at a variety of levels. If we can learn how these genes function in methanogenic *Archaea*, our overall understanding of the entire gene family will be greatly extended. In the long run, we may be able to improve the efficiency and the applicability of nitrogen fixation in agriculture.

9803553 Bacterial Dicarboxylate Transport in Symbiotic Nitrogen Fixation

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Grant 98-35305-6553; $100,000; 2 Years.

Crops such as soybean and alfalfa are unusual in being able to get the nitrogen they need to grow from symbiotic bacteria. These bacteria infect the roots and, together with the plant, form organs specialized for nitrogen production. This is no free lunch; the energy the bacteria use to convert inert atmospheric nitrogen into a form the plants can use comes from the plant, although the precise form in which it is transferred to the bacteria is unknown. What is known is that mutation in a bacterial protein that transports various dicarboxylic acids blocks nitrogen fixation. We have found that the specificity of this transport protein is broader than previously thought and will determine how wide a range of substrates it can recognize. In order to learn which of these potential substrates is most important in nitrogen fixation, we will try to modify the transport protein genetically to narrow the range of substrates it will use and determine the effect of this alteration on nitrogen fixation. The results will be important in understanding how plant and bacterial metabolism are coordinated and in modifying metabolism to increase nitrogen fixation by this kind of plant-bacterial symbiosis.

9803541 The Role of VNFG in the *vnf*-encoded nitrogenase from *Azotobacter vinelandii*

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Grant 98-35305-6685; $120,000; 2 Years

Nitrogen is often the limiting nutrient in both sustainable and mechanized agricultural systems. The enzyme nitrogenase is capable of converting atmospheric N_2 to the preferred nitrogen source, ammonium, and thus holds the potential for enhancing all agricultural practices. The goals of this proposal will be to investigate the role of a protein, VnfG, which is required for the proper processing of the vanadium-containing nitrogenase from the soil bacterium *Azotobacter vinelandii*. VnfG is responsible for the proper placement of the active site catalytic factor (FeV-co) responsible for the nitrogen-converting catalysis carried out by nitrogenase. A radioisotope of vanadium (^{49}V) will be used to follow the binding of FeV-co to VnfG and for its insertion into the vanadium nitrogenase. The conditions that allow the proper placement of the active site catalytic cofactor will be investigated. The specificity of VnfG for FeV-co will also be tested. The ability of VnfG to perform the insertion of the molybdenum-containing homolog of FeV-co into nitrogenase proteins will be studied.